

**Trigeminovascular nociceptive neurotransmission: a
microiontophoretic study**

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Abstract

Migraine is a common and disabling condition, affecting up to 15% of the population. This thesis sought to characterise certain aspects of the pharmacology of trigeminovascular nociceptive neurotransmission in neurons of the trigeminocervical complex (TCC) in the cat and also the rat ventroposteromedial thalamic nucleus (VPM). Electrical stimulation of the superior sagittal sinus (SSS) combined with the microiontophoretic ejection of L-glutamate were used to locate the cell bodies of neurons activated by trigeminovascular nociceptive input in the two regions. Agents were then microiontophoretically ejected onto these neurons to study their modulation of action potential firing.

To investigate a potential role for the thalamus in migraine, commonly used anti-migraine agents were microiontophoresed onto thalamocortical neurons within the VPM nucleus. Sodium valproate reversibly inhibited the response to SSS stimulation and L-glutamate ejection. The effects of GABA were also studied and found to have a similar action to valproate on trigeminovascular nociception. This appeared to involve activation of both GABA_A and GABA_B receptors. Naratriptan (a typical second generation triptan) similarly inhibited both responses. In addition to its action on serotonin 5-HT_{1B/1D} receptors, this inhibition was also mediated by activation of 5-HT_{1A} receptors. Ergometrine maleate had a similar effect to naratriptan. Propranolol (a β blocker) reversibly inhibited the response to SSS stimulation and L-glutamate ejection. This appeared to be through blockade of β adrenoceptors as it could be antagonised by co-ejection of isoproterenol. The selective β_1 antagonist atenolol had a similar action to propranolol while β_2 and β_3 antagonists were ineffective. These results suggest that neurons in the VPM may be a target for anti-migraine medications and a fruitful subject for further research.

The role of high threshold voltage gated calcium channels (VDCC) in trigeminovascular nociceptive neurotransmission by second order neurons of the TCC was also studied. The effects of selective peptide blockers of L-, N-, and P/Q-type VDCCs were examined. Microiontophoretic ejection of ω -Agatoxin IVa / TK (P/Q-), ω -conotoxin GV1a (L-) and calciseptine (N-type) reversibly inhibited neuronal firing in response to L-glutamate. This indicates an important post-synaptic role for VDCCs in regulating nociceptive neurotransmission within the TCC.

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Published Papers and Abstracts

Papers

Shields KG, Goadsby PJ. Propranolol modulates trigeminovascular responses in thalamic ventroposteromedial nucleus: a role in migraine? *Brain* 2005; 128: 86-97.

Shields KG, Storer RJ, Akerman S and Goadsby PJ. Post-synaptic high threshold voltage dependent calcium channels modulate trigeminovascular nociceptive neurotransmission in the trigeminocervical complex of the cat. *Neuroscience (in press)*

Abstracts

Shields KG, Storer RJ, Akerman S, Goadsby PJ. Post-synaptic high threshold voltage dependent calcium channels modulate trigeminovascular nociceptive transmission in the trigeminocervical complex. *Cephalalgia* 2003; 23: 726

Shields KG, Kaube H, Goadsby PJ. GABA receptors modulate trigeminovascular nociceptive neurotransmission in the VPM thalamic nucleus of the rat. *Cephalalgia* 2003; 23: 728

Shields KG, Goadsby PJ. Propranolol modulates trigeminovascular nociception in the ventroposteromedial nucleus (VPM) of the thalamus: a possible site of action? *Neurology* 2004; 62: A534

Shields KG, Goadsby PJ. Serotonin receptors modulate trigeminovascular nociception in the ventroposteromedial nucleus (VPM) of the thalamus: a possible site of action of the triptans? *Neurology* 2004; 62: A535

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Introduction

“I have prefaced these volumes with the names of my authorities. I have done so because it is, in my opinion, a pleasant thing and one that shows an honourable modesty, to own up to those who were the means of one’s achievements...”

Pliny The Elder

(From the preface to the “Natural History”)

Migraine: a common and debilitating condition

Migraine is very common neurological condition, though its exact prevalence is still a matter of debate. Methodological variables may account for the disparities observed between studies (Gobel et al., 1994; Henry et al., 1992; Launer et al., 1999; Stewart et al., 1992); however reasonable estimates for the prevalence of migraine (all types) is of the order of 15% for females and 6% for males. Alternatively the life-time prevalence may range from 12% to 33% of women and 4% to 22% of men, but this may be dependent on recall bias (Davidoff, 2002). Migraine without aura (MO) accounts for the largest proportion of sufferers (Russell et al., 1996), far exceeding the number who experience migraine with aura (MA) (between 18% and 36% of the total). The percentage of patients experiencing both types of migraine at some stage in their headache life-time may be between 8% and 42% (Davidoff, 2002). Interestingly migraine prevalence is significantly higher in neurologists; particularly those with a specialist interest in headache (Evans et al., 2003) (Table 1). The reason for this is not clear, though neurotic and perfectionist personality traits are probably not to blame – at least not for migraine (Silberstein et al., 1995).

Table 1: Prevalence (one year and life-time) of migraine in neurologists and neurologists with a specialist interest in headache disorders.

	Neurologists		Headache Specialists	
	1 Year (%)	Life-time (%)	1 Year (%)	Life-time (%)
Male	35	47	59	72
Female	58	63	74	82

Adapted from (Evans et al., 2003)

What is less debatable is the debilitating nature of migraine headaches (Menken et al., 2000). Migraines have a deleterious impact on work performance. In the following discussion some of the more pertinent theories on the pathogenesis of migraine will be examined. The wider body of evidence relating to the mechanisms of trigeminovascular nociception will also be reviewed.

Theories of Migraine Pathogenesis

Many theories on the pathogenesis of migraine have been proposed, but none have been able to explain all of the clinical features of this complex condition. Of all the theories, the belief that migraine arose from abnormal constriction and dilation of intra-cranial blood vessels (vasoconstriction was responsible for aura, while vasodilation resulted in pain) held sway for the greatest period. This was the orthodox belief until regional cerebral blood flow studies suggested that the patterns of flow changes were more complicated (Olesen et al., 1990). The discovery that intracranial vessel dilation can occur as a reflex response to nociceptive stimulation of the V₁ dermatome (May et al., 1998a; May et al., 2001) raised the intriguing possibility that vascular changes could be a natural response to pain, rather than a cause. In the following discussion some of the more relevant, current theories shall be briefly discussed.

The Genetics of Migraine

Though several possible susceptibility loci have been identified for migraine with and without aura, it is likely that migraine is a complex, polygenic, multifactorial disorder in which genetic factors interact with each other and also environmental influences (Ducros et al., 2002; Estevez and Gardner, 2004; Ferrari and Haan, 2001).

Studies examining the genetic basis of migraine are complicated by its heterogeneous nature. Objective clinical and diagnostic tests for the condition are also lacking. Familial Hemiplegic Migraine (FHM) is a relative exception, as this rare variant of migraine with aura is inherited in an autosomal dominant manner. Different phenotypes of FHM however are described, including “pure” FHM and a variant with additional cerebellar dysfunction. At present two FHM loci have been identified: a) FHM1 locus affecting the Ca_v2.1 (CACNA1A) gene on chromosome 19q13 (Ophoff et al., 1998); this gene encodes the α_{1A} pore forming unit of P/Q-type voltage dependent calcium channels and mutations of this gene account for up to half of patients with FHM (Estevez and Gardner, 2004) and b) FHM2 involving a mutation of the ATP1A2 gene on chromosome 1q23 encoding a Na⁺/K⁺ ATPase (De Fusco et al., 2003).

The first *in vivo* study to examine the effects of a CACNA1A mutation has shown enhanced neurotransmission at the neuromuscular junction, with reduced thresholds

for the triggering, and increased velocity of propagation, of cortical spreading depression (CSD) (van den Maagdenberg et al., 2004). Interestingly loss of CACNA1A and ATP1A2 function in mice may also lead to developmental abnormalities in the CNS, some of which involve noradrenergic neurons in the brainstem (Levitt and Noebels, 1981; Moseley et al., 2003). Further studies will be needed using whole animal preparations to establish the functional implications of these mutations. If these mutations are directly responsible for the pathogenesis of migraine it is unlikely that they would have effects confined to a single neural process, such as CSD. They would more likely result in a systemic dysfunction of neural functions at multiple levels and this may help explain the triggering and symptomatology of migraine.

Migraine involves a dysfunction of sensory processing: clinical and electrophysiological evidence

Undoubtedly pain is a prominent symptom of migraine. In contrast however with other primary headache disorders, such as cluster headache, with which it can share features of autonomic activation, sensory hypersensitivity is an essential diagnostic component of migraine (Davidoff, 2002). It typically manifests itself as either photophobia or phonophobia, or a combination of the two (Drummond, 1986; Vingen et al., 1998; Woodhouse and Drummond, 1993). Osmophobia may also be experienced (Blau and Solomon, 1985). This phenomenon of sensory hypersensitivity is much less prominent in other primary headache disorders, though it is not unknown, and is not reported following experimental head pain in humans (May et al., 1998a). This indicates that craniovascular pain is unlikely to be responsible for these symptoms.

If migraine was thought of as a disorder of sensory processing, and not just a disorder of head pain, the entire spectrum of sensory symptoms experienced during and between attacks could be explained. Any abnormality would probably necessarily involve the higher centres of sensory processing.

Visual and auditory stimuli may cause migraineurs discomfort even when headache free (Chronicle and Mulleners, 1996; Drummond, 1986; Vingen et al., 1998; Woodhouse and Drummond, 1993). Phonophobia is also a common symptom in migraineurs both between and during attacks (Main et al., 1997; Vingen et al., 1998;

Woodhouse and Drummond, 1993). There is a weak association between a lack of habituation of brainstem auditory evoked potentials (BAER) and lowering of thresholds for auditory discomfort (Sand and Vingen, 2000). This may indicate a central mechanism, especially as there is no evidence to suggest peripheral cochlear dysfunction in migraine (Woodhouse and Drummond, 1993).

The visual symptoms associated with the migrainous aura and headache - notably photophobia - are well recognised. Between headache attacks patients may also complain of “dazzle” (the painless exaggeration of brightness), sensitivity to glare or flickering light (Hay et al., 1994) and discomfort when looking at square-wave gratings (Chronicle and Mulleners, 1996; Chronicle et al., 1995; Marcus and Soso, 1989). Migraineurs report significantly greater visual discomfort when looking at square-wave gratings than healthy controls. They also report greater numbers of visual illusions when confronted by such patterns compared with healthy subjects (Coleston and Kennard, 1993; Marcus and Soso, 1989; Wilkins et al., 1984). Individuals suffering from migraine with aura (MA) are reported to display faster reaction times for tasks requiring “low-level” visual processing including simple tasks such as detecting the orientation of lines, the temporal sequence of the appearance of objects (Wray et al., 1995) and altered thresholds for target detection (Chronicle et al., 1995). Many studies examining this phenomenon have failed to differentiate between the two types of migraine (Drummond, 1986; Marcus and Soso, 1989; Wilkins et al., 1984; Woodhouse and Drummond, 1993), or only examined one type of migraine (Chronicle et al., 1995; Wray et al., 1995). In spite of this, differences in visual sensory processing undoubtedly exist between migraine patients and non-migraine control subjects both during and between attacks. A spectrum of visual dysfunction/symptomatology may exist between migraine patients with aura (MA) and those without aura symptoms (MO). Control subjects and MA patients may represent the two extremes of the spectrum, with MO patients lying between them (Chronicle and Mulleners, 1996; Chronicle et al., 1995; Coleston and Kennard, 1993; Khalil and Legg, 1989).

The basis for these visual abnormalities is not well understood. The same square-wave gratings that trigger discomfort or headache in migraine patients may also precipitate epileptiform activity in the EEG of individuals with photosensitive epilepsy (Wilkins et al., 1984). This has been taken to indicate that a dysfunction of cortical sensory processing – possibly due to defective GABAergic inhibitory

interneurons – is responsible (Chronicle et al., 1995; Wilkins et al., 1984; Wray et al., 1995). Assuming that the irides are free of pathology, photosensitivity may be associated with parenchymal lesions of the diencephalon, so-called “central dazzle” (Cummings and Gittinger, 1981; Riddoch, 1938) or even of the trigeminal nucleus (Gutrecht et al., 1990). Photophobia may be increased in migraineurs by nociceptive stimulation of the trigeminal nerve (Drummond and Woodhouse, 1993; Wolff, 1963) possibly reflecting functional disturbances at both brainstem and cortical levels (Wolff, 1963). It may therefore be more appropriate to take a broader perspective on the physiological basis of photosensitivity and sensory hypersensitivity in migraine. It may reflect a system-wide dysregulation of sensory information at multiple levels in the CNS

Neurophysiological abnormalities in migraine

Numerous studies have examined evoked and event-related potentials in migraineurs. These have been reviewed in depth by Ambrosini et al., 2003a; Giffin and Kaube 2002; Schoenen et al., 2003; Schoenen and Thomsen, 2000, and the following discussion is adapted in part from these papers. Electrophysiological studies provide insights into the sensory processing abnormalities that may exist in migraine. A prominent photic driving response on the electroencephalogram (EEG) was the first significant and consistently observed electrophysiological abnormality in migraineurs (Golla and Winter, 1959). Though this “H-Response” has little clinical use in migraine, it demonstrates that there are differences in cortical sensory evoked responses between migraineurs and normal control subjects.

A considerable amount of contradictory evidence exists concerning the amplitudes of sensory evoked cortical potentials in migraine patients (Connolly et al., 1982; Lehtonen, 1974; Polich et al., 1986). During repetitive stimulation, amplitudes are generally lower initially in migraineurs compared to normal subjects. They also appear to be lower during an attack (MacLean et al., 1975). Following repetitive stimulation evoked potential amplitudes increase in migraine patients whereas in non-migraine patients the usual response is for the amplitude to decrease or habituate (Schoenen et al., 1995; Wang and Schoenen, 1998; Wang et al., 1996). The terms “cortical hypoexcitability” or “decreased cortical preactivation” have been used to explain this phenomenon – but their precise meaning remains to be determined.

Evoked and event-related potentials (especially slow event related potentials such as contingent negative variation (CNV)) (Birbaumer et al., 1990; Rebert et al., 1986) recorded from the scalp probably reflect activity in both cortical and sub-cortical structures (for example medial thalamotomy significantly alters the amplitude of the cortical CNV (Tsubokawa and Moriyasu, 1978)). As the cortex and thalamus are functionally inextricably linked, it is not surprising that evidence is emerging to suggest that inter-ictal cortical abnormalities may be due to a dysfunction in thalamocortical projections (Vandenheede et al., 2003).

Other electrophysiological abnormalities in migraine patients include the intensity dependence of auditory evoked potentials (IDAP) (Maertens de Noordhout et al., 1986; Wang et al., 1996). IDAP (where the evoked potential augments in response to increasing auditory stimuli) is linked to abnormalities of habituation (Ambrosini et al., 2003b). There is a tendency towards abnormal IDAP responses in migrainous families, even in members who do not suffer from migraine (Sandor et al., 1999) and it normalises during a migraine (Judit et al., 2000). CNV or the “expectancy wave” (Knott and Irwin, 1973) on the other hand is an event related potential involving cognition and motor preparation. It has two components, an early component that is thought to indicate the level of expectation and this may be modulated by catecholamines. The late component is related to motor readiness and is thought to be modulated by dopamine. The amplitude of the first component in particular is greater in migraineurs (especially without aura) than controls.

To study further the vexed issue of “cortical excitability” transcranial magnetic stimulation (TMS) studies of the parietal and occipital cortex have been undertaken. TMS may be used as a means of directly and non-invasively assessing cortical “excitability” using intense magnetic pulses. Magnetic pulses are delivered to the cortex by electromagnetic coils. The nature of the magnetic pulse may however differ significantly between coils such as the circular and a “figure-of-eight” which is a potentially important confounding variable (Schoenen et al., 2003). The stimulation threshold at which responses such as the generation of phosphenes (flashes of light) or compound muscle action potentials are generated is then interpreted as indicating the level of “cortical excitability”. Studies using TMS stimulation of the motor cortex have yielded results that may demonstrate cortical “hypoexcitability” (Afra et al., 1998), “hyperexcitability” (Aurora et al., 1999a) or even no difference (Bohotin et al., 2003; Werhahn et al., 2000) between controls and migraine patients. In the occipital

cortex TMS again yields equally conflicting results with both cortical hyperexcitability (Aurora et al., 1998; Aurora et al., 1999b; Aurora et al., 2003), hypoexcitability or no discernible difference (Afra et al., 1998; Battelli et al., 2002; Bohotin et al., 2002; Brighina et al., 2002; Fierro et al., 2003). Methodological differences and the subjective nature of some of the measured outcomes (notably phosphene generation evoked by occipital TMS) may account for some of the discrepancies between studies. Unfortunately it is therefore difficult to proffer any firm opinions regarding levels of cortical “excitability” other than abnormalities certainly appear to be present in migraine patients but their precise nature remains to be determined. It would be especially useful if TMS studies, using objective and quantifiable outcomes such as visual evoked potentials (Bohotin et al., 2002), were performed at different stimulating frequencies using both circular and “figure-of-eight” coils and then comparing the effects. Only then will it be possible to establish if cortical “excitability” is truly altered in migraine sufferers.

Lack of habituation, or dishabituation, of sensory evoked and event related potentials however remains the most consistently observed of all the electrophysiological abnormalities in migraine sufferers (Schoenen et al., 1995). The habituating response appears to “normalise”, resembling the response observed in control subjects, before the onset of a migraine. It remains at normal levels for the duration of the migraine and for up to 2-3 days after an attack (Judit et al., 2000). This suggests that just as patients may have premonitory symptoms well before the onset of headache (Blau, 1980; Giffin et al., 2003), an underlying neurological dysfunction which is reflected in these electrophysiological abnormalities may precede the onset of the headache phase and be causally related to it.

Abnormal habituation may be indicative of low interictal central serotonergic activity (Hegerl and Juckel, 1993). Ablation of ascending midbrain 5-HT projections in rats results in behavioural changes including reduced habituation to touch and auditory stimulation (Geyer et al., 1976; Hole et al., 1977). Drugs with central serotonergic activity including SSRIs and triptans have the ability to correct IDAP, interictal dishabituation and motor responses following parietal TMS (Evers et al., 1997; Juckel et al., 1999; Juckel et al., 1997; Ozkul and Bozlar, 2002; Proietti-Cecchini et al., 1997; Werhahn et al., 1998). This normalisation of habituation has been paralleled by a clinical improvement in migraine (Ozkul and Bozlar, 2002).

CNV alternatively may be modulated by, amongst others, the central catecholaminergic systems. Treatment with β -blockers leads to decreases in CNV amplitudes (Maertens de Noordhout et al., 1987) and clinical outcomes following treatment with β -blockers are better in those with higher initial CNV amplitudes (Schoenen et al., 1986). Interestingly β -blockers also normalise IDAP (Sandor et al., 2000) and the amplitudes of visual evoked potentials (Diener et al., 1989) in migraineurs. Catecholamines may play an important role in modulating the GABAergic feed-forward and recurrent inhibitory circuits within and between the thalamus and cortex. Activity within these circuits may modify surface potentials. Modification of central GABA neurotransmission also modulates TMS responses. Sodium valproate treatment appears to raise the threshold for phosphene generation in the occipital cortex (Mulleners et al., 2002). Taken together this suggests that serotonin, GABA and noradrenaline modulate these electrophysiological responses and assuming that the responses do reflect an underlying brain dysfunction, these neurotransmitters may be intimately involved in the processes that lead to the migrainous state.

The significance of these electrophysiological abnormalities is far from clear however. It is perhaps optimistic to suggest that experimental electrophysiological observations, which reflect global responses of cortical and sub-cortical circuits, represent the neurophysiological properties (such as thresholds for membrane depolarisation) of cortical neurons. The fact that therapeutic agents, known to be clinically effective against migraine, may influence these electrophysiological findings provides circumstantial evidence for their relevance. Experimental evidence does support the theory that migraine represents a dysregulation of sensory neurotransmission at cortical and subcortical sites, but an exact explanation of the nature of this dysfunction remains elusive.

Cortical spreading depression and migraine aura

Spreading depression, characterised by widespread, rapid and almost complete neuronal depolarisation, is a dramatic response of the grey matter of the central nervous system to a variety of insults. This depolarisation occurs as an “all-or-none” type process that propagates in a wave-like manner through the grey matter at a constant rate of 4-6 mm/minute. It does not respect vascular territories and its spread

is only restricted by major architectural land-marks such as the central sulcus (Somjen, 2001). There is a massive redistribution of ions between the intra- and extracellular compartments. Extracellular levels of K^+ ions increase dramatically from 2.3 to 35mM, while Ca^{2+} , Cl^- and Na^+ all fall (Kraig and Nicholson, 1978). There is also a large release of amino acids, such as glutamate and aspartate (Fabricius et al., 1993) and catecholamines into the interstitial spaces (Pavlasek et al., 1993). Neuronal metabolism increases considerably and anaerobic glycolysis occurs. This is associated with an extracellular accumulation of lactate and H^+ ions (Scheller et al., 1992).

Following the initial depolarisation, electrophysiological perturbations associated with cortical spreading depression (CSD) may persist for up to 20-30 minutes. Major alterations of cortical blood flow however persist for considerably longer. In the rat CSD is associated with an initial short-lasting increase in cortical blood flow (CBF) mediated at least by calcitonin gene-related peptide (CGRP) and nitric oxide (NO) (Wahl et al., 1994), which is then followed by a longer (>1hour) oligoemic phase during which CBF falls to approximately 70% of baseline values (Lauritzen, 1984; Lauritzen et al., 1982).

The similarities between CSD and the progressive march of aura symptoms are obvious, but evidence to prove the causal link was initially scant. Though it becomes progressively more difficult to elicit CSD in the brains of higher mammals, it probably occurs in the human cortex, though attempts to trigger it in brain slices have been unsuccessful (McLachlan and Girvin, 1994). Spontaneous events akin to CSD have been recorded in humans (Mayevsky et al., 1996; Strong et al., 2002). Changes in cortical blood flow have been observed in aura just as in animal models of CSD. A brief, spreading wave of focal cortical hyperaemia, not restricted to a particular vascular territory, (Olesen et al., 1981a) precedes a long-lasting oligoemia (Andersen et al., 1988; Olesen et al., 1990). This oligoemic phase occurs in the hemisphere contralateral to the hemifield in which visual symptoms of aura developed (Cutrer et al., 1998; Lauritzen and Olesen, 1984). These findings have been replicated using functional magnetic resonance imaging (fMRI) techniques (Cao et al., 1999; Hadjikhani et al., 2001). Additionally magnetoencephalographic (MEG) signals accompanying CSD from gyrencephalic species (Bowyer et al., 1999) are similar to those recorded from the scalps of patients during spontaneous and evoked auras (Bowyer et al., 2001).

It is now generally accepted that CSD is probably responsible for the aura, but can it be used to explain the wider spectrum of events occurring during a migraine attack? “Cortical hyperexcitability”, be it as a result of reduced inhibition or increased neuronal excitability, could lead to CSD generation in response to environmental stimuli. Many of the electrophysiological studies that claim to demonstrate “cortical hyperexcitability” have been performed on patients with aura and often in those in whom migraine may be triggered by visual stimulation. Repetitive CSD reduces paired pulse inhibition in rat cortical slices – implying a selective reduction of intracortical inhibitory processes. If the migrainous brain had an impaired capacity to deal with abnormalities of ion homeostasis due to poor energy reserve, impaired energy metabolism or low levels of magnesium (Barbiroli et al., 1992; Boska et al., 2002; Welch et al., 1989) CSD could possibly lead to significant neuronal dysfunction and clinical symptoms beyond those associated with the migraine aura.

CSD induces *c-fos*-like immunoreactivity (*c-fos* IR) in the rat cerebral cortex (Herrera et al., 1993). Repeated CSD (Moskowitz et al., 1993) also induces *c-fos* IR in the ipsilateral trigeminal nucleus caudalis (TNC), principally in laminae I and II and its ventral aspect (Bolay et al., 2002; Moskowitz et al., 1993). This can be prevented by pre-treatment with sumatriptan. CSD also causes plasma protein extravasation (PPE) in the dura mater. Both PPE and *c-fos* IR induction in the TNC are dependent on an intact trigeminal system. This distribution of *c-fos* IR is also observed after nociceptive stimulation of intracranial vascular structures such as the superior sagittal sinus. This suggests that CSD activates trigeminal neurons conveying nociceptive information.

PPE occurs as part of a sterile dural inflammatory process that may be triggered by activation of trigeminal nerve endings. Electrical stimulation of the trigeminal ganglion (Markowitz et al., 1987) results in mast cell degranulation and platelet aggregation in dural capillaries (Dimitriadou et al., 1991). This inflammatory process may be nociceptive (Strassman et al., 1996) and can be prevented by abortive anti-migraine therapies such as sumatriptan (Markowitz et al., 1988; Moskowitz and Cutrer, 1993).

This mechanism could explain how cortical dysfunction may result in both sensory hypersensitivity and head pain. It is however significantly flawed in many respects. The most obvious problem is that aura symptoms, and by extension CSD, occur in only a minority of migraine patients (Rasmussen and Olesen, 1992; Russell et al.,

1996). There is no evidence that CSD occurs in migraine without aura (MO) patients. It is difficult to suggest that such a dramatic electrophysiological abnormality would be clinically silent. Even allowing for involvement of less “eloquent” regions of the cortex and the possibility that CSD can be restricted to distinct depths of the cortex (Richter and Lehmenkuhler, 1993) some symptoms would be expected. In any animal model of CSD, conscious subjects almost invariably develop some functional impairment (Koroleva and Bures, 1993). If it is argued MO patients experience CSD that is in some way qualitatively different to MA patients, generalisations about its pathophysiology are therefore not necessarily valid. The current weight of evidence indicates that MO patients do not develop CSD-like phenomena. Cerebral perfusion studies in general do not demonstrate the characteristic changes in MO patients - though one notable exception revealed *bilateral* hypoperfusion (Woods et al., 1994). This is quite different to the unilateral perfusion defect observed in MA patients (Lauritzen and Olesen, 1984; Olesen et al., 1981b) and animal models of CSD.

CSD may also not be nociceptive. It commonly triggers vegetative responses and not aversive behaviour in conscious rats (Koroleva and Bures, 1993). CSD induction of *c-fos* like immunoreactivity could represent an artefact of the triggering process. CSD triggered by application of potassium chloride (Moskowitz et al., 1993) may instead induce *c-fos* IR by hyperosmolar activation of trigeminal fibres (Ingvarlsen et al., 1997). CSD does not appear to alter spontaneous firing, or the response to noxious thermal and mechanical stimulation of dural responsive trigeminal neurons *in vivo* (Ebersberger et al. 2001). Furthermore there is no evidence that CSD occurs clinically with the frequency employed in these studies. Importantly it is also well recognised that patients may experience aura symptoms without subsequently developing headache. Aura therefore does not automatically lead to head pain. Additionally MA patients do not always develop aura symptoms in association with their headaches, indicating that patients may have a mixed MA and MO phenotype. This indicates that aura is probably an epiphenomenon of migraine.

CSD probably is responsible for the clinical symptoms of aura. This important phenomenon may provide insights into some elements of the pathogenesis of migraine. As aura however only affects a minority of migraine sufferers, and it may occur in the absence of other migrainous symptoms, CSD studies can only provide limited insights into the pathogenesis of migraine. Finally, if PPE occurs in humans (May et al., 1998b) it would probably be painful and could account for some

particular qualities of migraine pain such as mechanical hypersensitivity. Unfortunately specific plasma protein extravasation blockers such as CP122,288 (Roon et al., 2000) are not effective as acute migraine treatments. This obviously calls into question whether PPE plays an essential role in producing migrainous pain.

The role of the brainstem in migraine.

Electrical stimulation of the periaqueductal grey (PAG) can be used for the treatment of intractable somatic pain. A fascinating side effect of this procedure is the triggering of head pain in previously headache-free individuals. These headaches have many of the characteristics of migraine including: unilateral location, throbbing character, associated nausea and vomiting (Raskin et al., 1987; Veloso et al., 1998). Some cases also responded favourably to dihydroergotamine (Raskin et al., 1987). Similarly migrainous-type headaches may be triggered by a variety of pathological lesions involving the rostral brainstem (Goadsby, 2002; Haas et al., 1993).

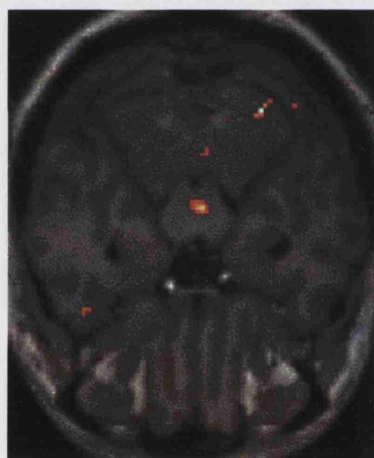


Figure 1: Brainstem activation during migraine: PET findings

Evidence of rostral brainstem activation during a spontaneous attack of migraine without aura. Perfusion changes are demonstrated in the dorsal rostral brainstem which were identified using positron emission tomography and are mapped on to a brain magnetic resonance image. Taken from (Bahra et al., 2001).

Compelling evidence is also provided by imaging studies of the region. Increased perfusion is demonstrated in the rostral brainstem using positron emission tomography (PET) during migraine attacks (Fig. 1) (Bahra et al., 2001; Weiller et al., 1995). These changes appear to be specific to migraine as they are not observed

during cluster headache or experimental head pain (May et al., 2000; May et al., 1998a). The brainstem perfusion changes persist following treatment with sumatriptan (and headache relief) unlike those in the cortex (Weiller et al., 1995). This makes it less likely that the changes are purely a reactive response to pain, something that has been confirmed by additional studies (May et al., 1998a). High resolution magnetic resonance imaging of the brainstem suggests that regional iron homeostasis (which may be an indicator of neuronal dysfunction) is selectively and progressively impaired in migraine with and without aura (Welch et al., 2001). Tissue iron values are higher than normal in patients with recent onset migraine in comparison with controls. There is also a positive correlation between duration of symptoms and degree of abnormality. This may indicate that brainstem dysfunction may precede the onset of migraine and is exacerbated by the disorder.

A further indication of the importance of the region is provided by studies in which P/Q-type voltage gated calcium channels (VDCC) were blocked. This leads to a facilitation of trigeminal nociceptive neurotransmission in the trigeminal spinal nucleus (Knight et al., 2002). This potentially provides a link between an ion channel dysfunction and the pathogenesis of head pain.

Unfortunately as the spatial resolution of PET scanning is limited it is not possible to define the specific structures responsible for these changes. In addition to the PAG other important monoaminergic modulatory centres such as the locus coeruleus (LC) and the rostral raphe nuclei are located within the area of interest. Nociceptive stimulation of intracranial dural structures results in enhanced *c-fos* IR in the LC, parabrachial nuclei, raphe nuclei and ventrolateral PAG (Ter Horst et al., 2001). These nuclei send projections to all parts of the neuroaxis. Electrical stimulation of the LC and the raphe nuclei alters cerebral blood flow (Goadsby et al., 1982; Goadsby et al., 1985). They are also part of an important anti-nociceptive system which is able to modulate nociceptive neurotransmission in the spinal dorsal horn (Basbaum and Fields, 1984).

The imaging findings however do not necessarily prove that this region acts as a migraine “generator”. To do so would require the demonstration of perfusion changes preceding the onset of headache symptoms and even then cause and effect would not be necessarily proven. It must also be remembered that in the largest series of patients experiencing head pain following PAG stimulation, headaches generally only developed after a latency of approximately two months (Veloso et al., 1998). This

suggests that electrical stimulation of the PAG has a dynamic effect on sensory neurotransmission, setting in train changes which can ultimately lead to a headache state. Why only some patients develop headache symptoms is not known. These patients however provide the best model for the *de novo* generation of migraine-like headache, replicating many of the clinical features of migraine.

The evidence suggesting that rostral brainstem structures have a central function in the pathogenesis of migraine is persuasive. Monoaminergic nuclei in this area modulate sensory neurotransmission at all levels of the neuroaxis. In addition to its well described anti-nociceptive function, the serotonergic system also facilitates the transmission of nociceptive information. A spino-bulbo-spinal pathway has an important role in the descending facilitation of nociceptive neurotransmission in the spinal dorsal horn (For a comprehensive review see Suzuki et al., 2004). This loop arises from nociceptive specific neurons in lamina I. These neurons express neurokinin I receptors and project to the dorsolateral pontine tegmentum and serotonergic neurons project in turn to the dorsal horn. The facilitatory action in the spinal dorsal horn appears to be mediated by activation of 5-HT₃ receptors (Suzuki et al., 2002). 5-HT₃ antagonists have antinociceptive actions in animal models of somatic pain, particularly in models of chronic inflammatory pain. They also have anti-nociceptive properties in models of visceral pain as well as having an analgesic effect in humans (McCleane et al., 2003). In addition to this descending pathway noradrenergic and serotonergic neurons (arising in the LC and rostral raphe nuclei) also have important ascending modulatory functions regulating arousal and attention in addition to nociceptive neurotransmission.

Though it may be simplistic to think of the brainstem as a site of an isolated migraine “generator”, abnormal neuronal activity in this region undoubtedly sets in motion a dynamic series of changes that can lead to a headache state in otherwise healthy individuals.

Central sensitisation

Characteristics of primary sensory afferent fibres

The following discussion is adapted from Doubell et al., 1999. Sensory neurons may be classified according to their axonal conduction velocities, axonal diameter and degree of myelination. Nociceptive afferents conduct sensory impulses in the A δ

(thinly myelinated) or C (unmyelinated) fibre range (Table 2). Somatic A δ and C fibre activation results in “first” and “second” pain respectively, referring to the immediate and delayed pain response following noxious stimulation. A δ fibre activation produces a sharp pain or a pricking sensation, while C fibre activation causes a dull ache or burning pain. The transduction of nociceptive sensory stimuli into electrical impulses is accomplished by several mechanisms, including the activation of G-protein coupled membrane receptors.

Small diameter afferent fibres in both somatic and visceral tissues fire in response to any number of noxious stimuli including trauma, heat and chemical irritation. An inflammatory response ensues which is accompanied by the release of intracellular contents from injured cells and also from specific inflammatory cells such as macrophages, lymphocytes and macrophages. In addition sensory nerves release peptides such as substance P (SP), calcitonin gene-related peptide (CGRP) and neurokinin A (NKA) from their peripheral terminals. These peptides change the excitability of sensory nerve fibres, produce vasodilation with associated leakage of intravascular proteins and also trigger the release of inflammatory mediators from leukocytes. This cascade of events leads to peripheral sensitisation of nociceptors. This results in tactile allodynia (when normally innocuous stimuli generate pain-associated behaviour) and increased responsiveness to thermal stimuli at the site of injury. These changes may be observed in primary trigeminal neurons innervating the dura mater (Strassman et al., 1996; Ebersberger et al., 1997; Schepelmann et al., 1999) Prolonged activity of peripheral C fibres can trigger central sensitisation – a neuroplastic phenomenon which produces long-lasting facilitatory changes in second (and perhaps third) order neurons, independent of input from primary afferent fibres (Woolf, 1983). The cardinal features of central sensitisation include:

- a) A reduction in the threshold for firing of nociceptors
- b) increased spontaneous firing rates of second order neurons
- c) increased responses of second order neurons to nociceptive C-fibre mediated inputs
- d) There is also expansion of receptive field size and responsiveness to previously innocuous (A β fibre input) stimuli (Woolf and King, 1990).

The NMDA receptor is central to the functional plasticity associated with central sensitisation. Sustained, repetitive firing of C fibres summate both spatially and temporally, generating slow post-synaptic potentials in second order dorsal horn

neurons (due to activation of AMPA, metabotropic glutamate and neurokinin 1 receptors). This removes the magnesium block of the NMDA receptor ion channel. Influx of calcium ions either through the NMDA receptor or through voltage gated calcium channels (VDCCs) activates protein kinase C (PKC). PKC phosphorylates serine/threonine residues on the NMDA receptor. As a result the magnesium block at resting membrane potentials is reduced, leading to long-lasting changes such as increased neuronal excitability and synaptic efficacy. NMDA ion channels may also be phosphorylated by other enzymes such as the tyrosine kinases TrkB – the high affinity receptor for brain derived nerve growth factor (BDNF).

Table 2: Characteristics of primary afferent sensory fibres

Fibre type	A β	A δ	C
Diameter (μ m)	>10	2-6	0.4-1.2
Conduction velocity (m/sec)	30-100	12-30	0.5-1.2
Myelination	Thickly myelinated	Thinly myelinated	Unmyelinated
Activation threshold	Low	High	High
Principal neurotransmitters released	EAA	SP, NKA, CGRP, EAA	SP, NKA, CGRP, EAA
Receptors activated post-synaptically	AMPA	NK ₁₋₃ , CGRP ₁₊₂ , NMDA/AMPA, mGLU	NK ₁₋₃ , CGRP ₁₊₂ , NMDA/AMPA, mGLU
Laminae in which neurons terminate	IIi, III, IV, V	I/Ilo, V	I/Ilo, V, X
Types of second order neurons contacted	LT, WDR	NS, WDR, LT	NS, WDR
Sensory modality transmitted	Innocuous: light touch, vibration and pressure	Noxious: sharp, pricking pain Sharp, stinging pain "First pain"	Noxious: thermal, mechanical and chemical irritation Dull, burning pain "Second pain"

AMPA: α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid, CGRP: calcitonin gene-related peptide, EAA: excitatory amino acids, LT: light touch, mGLU: metabotropic glutamate, NK: neurokinin, NKA: neurokinin A, NMDA: *N*-methyl-*D*-aspartic acid, NS: nociceptive specific, SP: substance P, WDR: wide dynamic range

Adapted from (Millan, 1999).

Central sensitisation in migraine: experimental and clinical correlates

An important observation is that such changes are observed in rat trigeminal nucleus caudalis neurons receiving convergent dural and cutaneous inputs (Burstein and Jakubowski, 2004; Burstein et al., 1998; Levy et al., 2004). Central sensitisation develops within 20 minutes of the application of an inflammatory “soup” to the dura (Burstein et al., 1998). Central sensitisation may be blocked by early treatment with sumatriptan (Burstein and Jakubowski, 2004; Levy et al., 2004). Once central sensitisation is established sumatriptan (like lignocaine) reverses the changes in dural receptive field size and responses to mechanical stimulation, but drug treatment has no effect on spontaneous neural firing or the enhanced responses triggered by stimulation of the cutaneous receptive field. This suggests that triptans act primarily on first order neurons and are only effective in treating migraine pain if given early, before central sensitisation becomes established (Burstein and Jakubowski, 2004; Burstein et al., 1998).

These animal studies can be correlated with results from clinical and electrophysiological studies. Patients display an enhanced R2 component of the blink reflex during a migraine headache (Kaube et al., 2002). This nociceptive component of the reflex is substantially larger than the same R2 elicited in the headache-free state. This may represent enhanced responsiveness of trigeminal neurons secondary to central sensitisation. Cutaneous allodynia may also be detected in patients during migraine attacks (Drummond, 1987) and may be a clinical sign of central sensitisation involving second or third order neurons (Burstein et al., 2004; Burstein et al., 2000a; Burstein et al., 2000b). Once cutaneous allodynia develops it is assumed that central sensitisation has taken place. Once this has occurred, the ability of triptans to alleviate the pain of migraine may be reduced (Burstein et al., 2004). Retrospective analysis of several clinical trials suggest that early treatment with triptans, while the headache is still mild, is associated with a better clinical outcome (Halpern et al., 2002; Hu et al., 2002; Pascual and Cabarrocas, 2002). Administration of triptans may prevent the development of central sensitisation and this has been proposed as the mechanism to explain this retrospective observation.

Another feature of central sensitisation is that it may also be associated with a change in the character of the head pain. Throbbing headache may be due to stimulation of sensitised primary afferent fibres. With the onset of central sensitisation the pain

becomes more constant and may be a reflection of increased spontaneous activity of second order neurons. Once central sensitisation had developed, only throbbing head pain was alleviated by triptan therapy (Burstein et al., 2004). It is argued that cutaneous allodynia does not represent a generalised dysfunction of sensory neurotransmission as only a minority of patients developed diffuse allodynia involving all four limbs (Burstein et al., 2000b). In those cases where it did occur it was argued that this was due to sensitisation of third order thalamocortical neurons.

Unfortunately a significant percentage of patients do not develop cutaneous allodynia during migraine attacks (Burstein et al., 2000b). As it is not detected during all migraines, central sensitisation cannot be used as an explanation for the development of headache in all migraine patients. Importantly enhancement of the R2 component of the blink reflex is not observed in patients suffering from headache secondary to frontal sinusitis (Katsarava et al., 2002). This painful condition involves nociceptive activation of the ophthalmic division of the trigeminal nerve and would be expected to trigger central sensitisation. This experimental paradigm however demonstrates qualitative differences between the two clinical conditions. Obviously this could be because the R2 component does not actually measure trigeminal sensitisation. Alternatively sensitisation may occur in both situations, but some additional neuromodulatory sensory dysfunction is required to generate a migraine attack.

Central sensitisation most likely occurs as a natural consequence of C fibre activation during a migraine attack. It potentially explains why early triptan therapy is more effective in some individuals, but it unfortunately does not explain the causes of migraine, nor explain clinical features such as photo- or phonophobia.

Innervation of intra-cranial dural structures

Dura mater

The dura mater is composed of two layers. The inner layer is folded to form the dural reflections such as the falx cerebrii and tentorium cerebellii. The outer endosteal layer adheres to the periosteum of the cranial cavity. The large venous sinuses are found between the two layers.

Light and electron microscopic examination of the dura mater and vessels reveals a plexus of A δ and C-fibres largely from the trigeminal (V) ganglion. A majority of these nerves have free unencapsulated nerve endings (Andres et al., 1987). These are

found throughout the dura mater but are particularly dense around the blood vessels, especially the superior sagittal sinus (Messlinger et al., 1993). These nerve fibres are immunoreactive for calcitonin gene-related peptide (CGRP), Substance P (SP) and neurokinin A (NKA) (Uddman and Edvinsson, 1989). The supratentorial dura mater is unequally innervated by the three divisions of the trigeminal nerve. Midline structures including the falx cerebri, in which the superior sagittal sinus is located, and tentorium cerebelli are innervated by fibres in the first, ophthalmic division (V_I). The floor of the anterior and middle cranial fossae are supplied by the maxillary (V_{II}) and mandibular (V_{III}) branches respectively (Steiger and Meakin, 1984). The posterior fossa dura is innervated by branches from the upper cervical segments (C_{1-2}), the vagus (X) and glossopharyngeal (XI) nerves.

Intra-cranial vascular structures

The intracranial vessels, both pial and dural, are similarly innervated by the trigeminal nerve. The superior sagittal sinus (SSS) is innervated by branches of the ophthalmic (V_I) nerve. The ventral third is supplied by the ethmoidal and the remainder is supplied by the tentorial nerve (Penfield and McNaughton, 1940). Studies, performed after unilateral application of horseradish peroxidase tracer, demonstrate a large projection to the V_I portion of the ipsilateral trigeminal ganglion. A smaller projection to the contralateral ganglion was also noted. Whether this represents a true bilateral sensory innervation of the SSS, or a result of diffusion of tracer across the midline is not clear (Mayberg et al., 1984). The middle meningeal artery and the circle of Willis, including its proximal branches and the terminal basilar artery, are innervated predominantly by V_I , with lesser contributions made by the V_{II} and V_{III} trigeminal divisions (Arbab et al., 1986; Mayberg et al., 1984; O'Connor and van der Kooy, 1986). Sectioning of the ipsilateral V_I nerve totally removes CGRP-like immunoreactivity on that side of the circle of Willis (Nozaki et al., 1990). While few sensory afferents have collateral branches from blood vessels and cutaneous areas, vascular and cutaneous afferents from the V_I dermatome are closely associated within the trigeminal ganglion (O'Connor and van der Kooy, 1986). Presumably these afferents project in continued close association to the trigeminal brainstem nuclear complex.

Autonomic innervation of intra-cranial vascular structures

The intra-cranial vasculature also receives a dense autonomic innervation. Parasympathetic fibres arising from the pontine superior salivatory nucleus (SSN) run in the facial nerve. They synapse in the pterygopalatine and otic ganglion from which the post-ganglionic fibres project to the cranial vasculature. Activation of this circuit may explain the autonomic features such as facial flushing, periorbital oedema and ptosis which may be experienced during migraine (Drummond et al., 1983; Gibbins et al., 1984; Suzuki et al., 1988). There is in addition a sympathetic innervation arising from the hypothalamus. This is relayed through the superior cervical ganglion before the nerves enter the cranial cavity as a plexus of fibres surrounding the internal carotid artery (Edvinsson and Goadsby, 1995).

The Trigeminal Brainstem Nuclear Complex

The sensory root of the trigeminal nerve enters the lateral pons before terminating in the trigeminal nucleus (Fig. 2a,b). The sensory trigeminal nucleus is not an homogeneous unit but a complex divided into the principal (Vp) and spinal nuclei (Vsp). A very large proportion of tactile trigeminal afferents, especially from the vibrissae in rats, terminate in the Vp. Other afferents, mainly nociceptive, descend in the spinal trigeminal tract to the Vsp. The spinal nucleus is further divided into the nucleus oralis (Vo), interpolaris (Vi) and caudalis (Vc). These are arranged in a rostro-caudal order (Olszewski, 1950).

The Vc, also known as the medullary dorsal horn (MDH), is a continuation of the spinal dorsal horn. The architectural structure of the dorsal horn is modified as it comes to lie in the concavity of the trigeminal tract. It increases in size and can be divided into several regions (corresponding approximately to the laminae of the spinal dorsal horn). The outermost layer, the subnucleus marginalis corresponds to lamina I. Deeper or ventral to this is found the subnucleus gelatinosus (lamina II) while the nucleus magnocellularis corresponds to laminae III and IV. The subnucleus reticularis dorsalis of the medullary reticular formation, which is equivalent to lamina V, is however not as clearly defined in Vc as lamina V in the spinal dorsal horn (Olszewski, 1950).

Primary afferent sensory fibres converge on second-order neurons in the medullary dorsal horn. Second order neurons can be classified into three categories according to

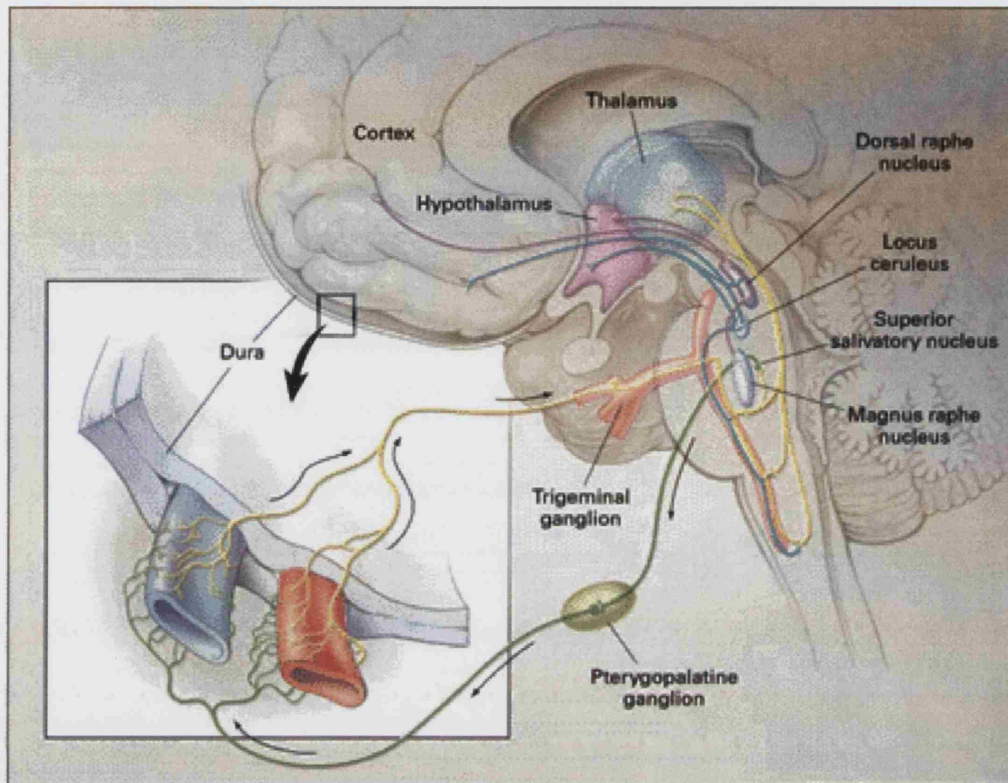


Figure 2a: Peripheral and central trigeminovascular nociceptive pathways

Anatomy of the main structures involved in trigeminovascular nociceptive neurotransmission. Sensory fibres innervating the cranial vessels arise from neurons which have their cell bodies within the trigeminal ganglion. Sensory inputs from the dural blood vessels (such as the superior sagittal sinus and middle meningeal artery) synapse on second order neurons in the trigeminocervical complex. These in turn project to the thalamus in the quinto/trigemino-thalamic tract. There are also connections between pontine neurons and the superior salivatory nucleus which results in a reflex activation of parasympathetic fibres (with resultant vasodilation). These parasympathetic neurons relay in the pterygopalatine ganglion.

Taken from (Goadsby et al., 2002b).

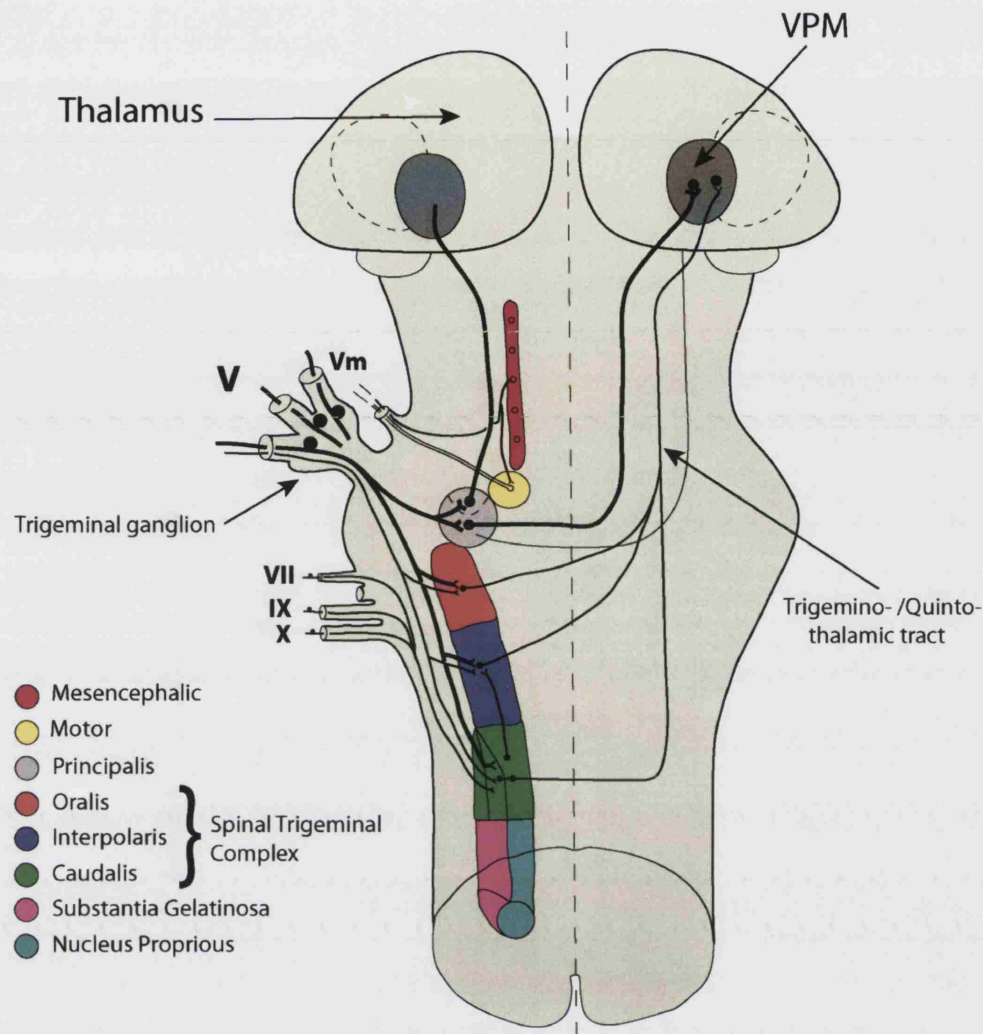


Figure 3b: Peripheral and central trigeminovascular nociceptive pathways

The three divisions of the trigeminal (V) nerve converge at the trigeminal or Gasserian ganglion to form the nerve root. This enters the lateral aspect of the pons and nociceptive fibres descend in the trigeminal tract to the caudal part of the spinal trigeminal nucleus (Vsp). Nociceptive fibres are also relayed in the other subdivisions of Vsp. The subdivisions of Vsp appear to be interconnected and may modulate activity in each other. Nociceptive second order neurons in Vsp project via the trigemino/quinto thalamic tract to the VPM nucleus of the thalamus. The facial (VII), glossopharyngeal (IX) and vagus (X) nerves also convey sensory fibres from the tympanic membrane and external auditory canal to Vsp. (V:trigeminal nerve; Vm: motor root of the trigeminal nerve; VPM: ventroposteromedial nucleus of the thalamus; VII: facial nerve; IX: glossopharyngeal nerve; X: vagus nerve)

their sensory responses (Hu, 1990; Hu et al., 1981). Nociceptive-specific (NS) neurons are activated by high intensity, noxious stimulation sufficient to cause tissue damage. These neurons are generally silent unless activated by a noxious stimulation and receive inputs from C fibres. Wide-dynamic range (WDR) neurons are activated by both innocuous and noxious stimulation. They exhibit an incremental response to mechanical stimuli, which varies proportionately with stimulus intensity. An important feature of WDR second order neurons is they receive considerable convergent inputs from extracranial cutaneous and intracranial visceral (including vascular and dural) structures. They may respond to a wide variety of stimuli (such as mechanical and chemical) which activate A β , A δ and C fibres. Viscerosomatic convergence, whereby first order afferents from visceral and somatic structures converge on the same second order neuron, is common in the spinal and medullary dorsal horn (Cervero, 1994; Sessle et al., 1986). Second order neurons which are responsive to intracranial vascular stimulation will often also respond to nociceptive stimulation of cutaneous receptive fields located in the V₁ territory, though other craniofacial receptive fields are frequently observed (Burstein et al., 1998; Davis and Dostrovsky, 1988c; Schepelmann et al., 1999; Strassman et al., 1986). This provides a possible mechanism to explain the pattern of referred pain experienced following electrical stimulation of the intracranial blood vessels. A third class is the non-nociceptive low-threshold (LT) neurons which respond only to innocuous stimulation, such as light touch.

Clinical and electrophysiological evidence for nociceptive neurotransmission in the trigeminal nucleus caudalis

Damage to Vc, as might occur as part of the lateral medullary syndrome (Wallenberg's syndrome) or syringobulbia, leads to the clinical finding of dissociated anaesthesia (Bradley et al., 2000). Pain perception in the face is impaired but light touch is preserved. This indicates clinically that trigeminal pain and thermal sensation, but not light touch, is relayed principally through Vc.

Intracellular labelling of functionally characterised nociceptive (both NS and WDR) neurons in Vc indicates that primary afferents terminate in laminae I-II (NS) and IV-V (WDR). Immunoreactivity for calcitonin gene-related peptide (CGRP) and substance P (SP) is found in these laminae (Boissonade et al., 1993; Henry et al., 1996; Jacquin

et al., 1986; Pearson and Jennes, 1988) Similarly immunoreactivity for the protein product of the immediate-early gene *c-fos* is also demonstrated in these laminae after nociceptive stimulation of both intracranial and extracranial structures (Strassman et al., 1993). Though multiple electrophysiological studies have consistently revealed trigeminal nociceptive neurons in laminae I-II and V (Hu et al., 1981; Price et al., 1976; Sessle et al., 1986) nociceptive neurons with dural receptive fields (RF) are more often recorded in lamina V (Burstein et al., 1998; Davis and Dostrovsky, 1986; Davis and Dostrovsky, 1988c; Schepelmann et al., 1999). This may simply reflect a bias due to the greater technical difficulties associated with recording from small neurons in lamina I and II.

Within the medulla there is a double somatotopic representation of the head and face (Strassman and Vos, 1993; Yokota and Nishikawa, 1980). Craniofacial structures are represented in a ventro-dorsal and rostro-caudal direction. The ventro-dorsal somatotopy has the ophthalmic representation placed ventrally, the mandibular dorsally and the maxillary representation lying in between. This is also the case for the other subnuclei (Shigenaga et al., 1986; Strassman and Vos, 1993). As would be predicted from this topographic arrangement, neurons responsive to intracranial dural stimulation are located in the ventrolateral aspect of Vc (Burstein et al., 1998; Davis and Dostrovsky, 1986; Schepelmann et al., 1999). The rostro-caudal representation is organised so that the most rostral structures are arranged at the level of the obex. Descending caudally the receptive fields represented gradually shift to the periphery of the face. The dermatomes of the cervical segments are the most caudal (Yokota and Nishikawa, 1980). This “onion-skin” arrangement tallies with clinical experience. Ascending damage by a syrinx results in a “balaclava” sensory loss which progresses concentrically from the periphery of the face towards the nose (Bradley et al., 2000).

Pain results from intra-cranial vascular stimulation in humans

From a translational neurosciences perspective, pain (nociception) is the sensory modality most amenable to study when investigating the pathophysiology of migraine. The fundamental rationale for all studies modelling intracranial nociception derives from the clinical experiences of patients undergoing neurosurgical operations. Observations made from several studies (Feindel et al., 1960; Penfield and McNaughton, 1940; Ray and Wolff, 1940; Wirth and Van Buren, 1971) demonstrate

that pain may be elicited from several intracranial structures. These may be summarised as follows:

- a) Dura mater: the supratentorial dura covering the cerebral convexities is poorly sensitive to stimulation (cutting, burning, electrical or mechanical stimulation) except for the dura along the margins of the venous sinuses and middle meningeal artery. This is also the case for the dura covering the floor of the middle cranial fossa and also the convexities of the cerebellar hemispheres. In contrast the floor of the anterior fossa is sensitive to electrical stimulation (resulting in pain referred to the periorbital region of the ipsilateral eye) as is the floor of the posterior fossa (referred to the retroauricular and suboccipital area). Mechanical stimulation produced inconsistent results. The tentorium cerebelli appears to be sensitive only on its rostral surface (causing pain in the ipsilateral frontal and periorbital regions).
- b) The dural venous sinus: the superior sagittal sinus is extremely sensitive to all forms of stimulation. This results in pain which can be described as being sharp, pressing or “headache-like”. It is felt in the ipsilateral periorbital and frontal regions and can be accompanied by vegetative symptoms including yawning, restlessness, nausea and vomiting. The other venous sinuses (straight sinus, torcular Herophili and transverse sinus) are also very sensitive to all forms of stimulation.
- c) Dural arteries: the middle meningeal (MMA) is exquisitely sensitive to all forms of stimulation (including distension) along its entire length. The pain is deep, aching and localised in the temporo-parietal area.
- d) Pial arteries: stimulation of the proximal segments of the major pial arteries (intracranial segment of the internal carotid, middle cerebral artery and anterior cerebral artery) results in an intense, diffuse, dull, aching pain. After repeated stimulation the pain becomes throbbing with associated nausea. Again it is centred on the peri-orbital and retro-orbital areas.

The pain following SSS and MMA stimulation in humans is visceral in nature i.e. it is difficult to precisely describe its origin or character and it is associated with autonomic features in particular nausea (see below). This is very similar to migraine pain. It is reasonable to assume that such stimulation is also nociceptive in other species and that it may activate the same sensory pathways involved in the perception of pain during migraine.

Migraine and visceral pain

The following summary is adapted from the following reviews: Cervero, 1994; Cervero, 2000; Cervero and Janig, 1992; Cervero and Laird, 1999.

Visceral pain is perhaps the most common form of pain produced by disease. It is clinically very different to somatic pain, even when referred to cutaneous sites. Most internal organs rarely generate any sensation apart from discomfort or pain. The principal features distinguishing visceral from somatic pain include:

- a) visceral pain may not be linked to actual visceral injury.
- b) visceral pain is often referred to other sites.
- c) visceral pain is poorly localised, diffuse and often deep seated in nature, but not all viscera (such as the lung and brain parenchyma) generate visceral pain.
- d) motor and autonomic reflexes (such as nausea and sweating) often accompany visceral pain.

The pain associated with migraine has all of the characteristics of visceral pain. Patients are often not able to accurately describe or locate the focus of their pain; only that it is deep and often throbbing. Our present understanding of pain processing is mainly derived from studies of somatic pain but it should not be assumed that the visceral and somatic nociceptive systems function identically (Strigo et al., 2003).

Visceral receptors appear to belong to three groups. The first are receptors which only respond to and encode the intensity of visceral stimulation in the noxious range. The second group are receptors that are able to encode the intensity of stimuli, ranging in a continuous spectrum from innocuous to noxious. These intensity-encoded receptors are activated by physiological stimuli. The final group are the “silent” nociceptors. These receptors are normally unresponsive, only becoming active in the presence of tissue inflammation. In the absence of inflammation noxious visceral events are encoded mainly by the high-threshold and intensity-encoded receptors.

Most visceral afferent fibres terminate in lamina I and V of the spinal cord just like somatic nociceptive fibres. They often express peptide neurotransmitters such as SP and CGRP. Visceral nociception however may not be transmitted by the classic spinothalamic (STT) and spinoreticular (SRT) tracts. Three alternative pathways have been discovered: a) the dorsal column pathway, b) the spino/trigemino-parabrachio-amygdaloid pathway, c) spino-hypothalamic pathway.

At the level of the thalamus, a majority of cells in the ventrobasal (VB) complex display viscerosomatic convergence. This fact, combined with evidence of thalamic

activation in many functional imaging studies indicates an important role for the thalamus as a relay for visceral nociception. The thalamus may have a special role in regulating the flow of visceral nociceptive information to the cortex. It may act as a “gate” to regulate the conscious appreciation of visceral pain (Rosen et al., 1994; Rosen et al., 1996). There is no functional imaging evidence of an exact somatic equivalent (Ladabaum et al., 2000).

Trigeminovascular nociception in the “trigemincervical complex”

It has been widely accepted that the intracranial blood vessels have a role in the pathogenesis of migraine (Fig. 2). As already discussed electrical stimulation of the superior sagittal sinus is painful in humans and it also results in release of CGRP into the cranial circulation (Zagami et al., 1990). This occurs in humans following thermocoagulation of the trigeminal ganglion and during spontaneous attacks of migraine (Goadsby et al., 1988; Goadsby et al., 1990). Electrical stimulation of the SSS is therefore considered to be a reasonable model for trigeminovascular nociceptive activation. It may replicate some of the pain components of a migraine attack.

Electrical stimulation of the SSS produces increased metabolic activity and regional blood flow in Vc along with other areas such as the frontal and parietal cortices (Lambert et al., 1988). It also increases metabolic activity and regional blood flow in the dorsolateral spinal cord at the level of the second cervical segment (Goadsby and Zagami, 1991). This increase in metabolic activity is due to trigeminovascular nociceptive neurotransmission as destruction of the trigeminal ganglion prevents these changes. Metabolic studies using uptake of radioactive tracers unfortunately have relatively poor anatomical resolution. This may be overcome by examining the distribution of immunoreactivity for the immediate early gene product *c-fos*. Examining the distribution of *c-fos* IR expression following SSS stimulation has the advantage of visualising almost the entire population of neurons activated by this stimulus. Again evidence of activation is found in Vc and the upper cervical dorsal horn in several species including rat, cat and monkey (Goadsby and Hoskin, 1997; Kaube et al., 1993b; Strassman et al., 1994). As a result the dorsal horn of the upper cervical cord may be considered to be a functional extension of Vc. The dorsal horn

of the first two cervical segments and Vc are therefore often collectively referred to as the trigeminocervical complex (TCC).

Expression of *c-fos* IR is localised in laminae I and the outer part of II, V and X of the MDH (in addition to other structures such as the nucleus of the solitary tract). Distinct differences in *c-fos* IR are observed when comparing the effects of nociceptive stimulation of vascular versus cutaneous structures (Strassman et al., 1994). Vascular stimulation tends to generate diffuse *c-fos* IR in contrast to the much more localised pattern observed following cutaneous stimulation. It extends from the boundary between Vi and Vc to C₂. Within the dorsal horn *c-fos* IR is found along the entire ventrolateral to dorsomedial axis, roughly corresponding to the somatotopic representation of the dorsal half of the face and head. This expression of *c-fos* IR is dependant on glutamate neurotransmission (Classey et al. 2001; Mitsikostas et al., 1999b; Mitsikostas et al., 1998) and can be effectively blocked by pre-treatment with migraine abortive agents such as sumatriptan and dihydroergotamine (Hoskin et al., 1996a; Hoskin et al., 1996b). This supports the view that trigeminovascular nociception is relayed through the TCC and that this may be a target for anti-migraine drugs.

L-Glutamate is probably the main excitatory neurotransmitter involved in nociceptive neurotransmission in the trigeminocervical complex. Noxious stimulation of the face results in the release of glutamate and aspartate in Vc (Bereiter and Benetti, 1996). High densities of NMDA, AMPA, kainate and metabotropic glutamate-type receptor binding sites are found in the superficial laminae of the complex. Ample evidence indicates that both NMDA and non-NMDA glutamate-type receptors are involved in nociceptive neurotransmission (Furuyama et al., 1993; Storer and Goadsby, 1999; Tallaksen-Greene et al., 1992).

Trigeminovascular nociception may be modulated by multiple mechanisms within the trigeminocervical complex. This has lead to a reappraisal of our understanding of how anti-migraine drugs may act. The most important involves the triptans – serotonin 5-HT_{1B/1D} receptor antagonists. Though still a contentious subject, there is histological and electrophysiological evidence to support a role for 5-HT_{1A,1B,1D} and 5-HT_{1F} (Boers et al., 2000; Mitsikostas and Sanchez del Rio, 2001; Storer and Goadsby, 1997) receptors in the pre- and post-synaptic modulation of trigeminovascular nociception. The response of second order TCC neurons following trigeminovascular nociceptive stimulation is suppressed by descending modulatory inputs from the nucleus raphe

magnus (NRM) (Lambert et al., 2001). This can be mimicked by 5-HT_{1B/1D} agonists. Triptans however do not have general anti-nociceptive properties as they have no effect on spinal transmission of pain (Cumberbatch et al., 1998). This may be due to the differential modulatory effects of 5-HT_{1B} and 5-HT_{1D} receptors on visceral trigeminovascular versus cutaneous nociception. 5-HT_{1D} receptors may be more important in trigeminovascular nociception, while 5-HT_{1B} receptors may be more important in somatic nociceptive neurotransmission (Donaldson et al., 2002). Interestingly 5-HT_{1D} receptor expression on the central terminals of primary afferents appears to be dynamically regulated (Ahn et al., 2004). Receptor expression on plasma membranes is very low at baseline but it is upregulated following nociceptive stimulation. This may explain why triptans are less effective if given too early during an attack. Other receptor systems with demonstrable modulatory functions in the spinal dorsal horn include GABA_A and GABA_B receptors, μ opioid receptors and adenosine A₁ receptors (Goadsby et al., 2002a; Storer et al., 2001; Storer et al., 2003).

Nociceptive neurotransmission in other regions of the trigeminal nuclear complex

Nociceptive neurons are not confined exclusively to the trigeminocervical complex. Electrophysiological evidence points to populations of nociceptive neurons in the other trigeminal subnuclei (Dallel et al., 1990; Davis and Dostrovsky, 1988c; Hayashi et al., 1984; Hu et al., 1981; Jacquin et al., 1988; Westrum et al., 1981). Even following section of the trigeminal brainstem nuclear complex at the level of the obex, nociceptive stimulation of facial receptive fields can still activate thalamic neurons (though at this level the border between Vc and Vi is indistinct, and a population of nociceptive responsive neurons may have been left intact) (Raboisson et al., 1989). Immunoreactivity for CGRP is found in Vp, Vo and Vi indicating that all these nuclei may have a role in trigeminal nociceptive processing (Henry et al., 1996; Tashiro et al., 1991).

There is also ample anatomical evidence for intra-trigeminal projections, linking the various subnuclei. Vp receives projections from all of the trigeminal subnuclei and cells arising from Vc project to all of the rostral trigeminal centres (Jacquin et al., 1990; Stewart and King, 1963). Cells in Vc can also be antidromically activated by stimulation of neurons in Vo (Hu and Sessle, 1979). This interconnecting system may

modulate the activity of trigeminal neurons. Injection of opiates into Vc can inhibit the responses of nociceptive responsive neurons in Vo, while morphine has no effect when directly injected into Vo (Dallel et al., 1998). It may therefore be an oversimplification to think of the trigeminal brainstem nuclear complex as a series of functionally distinct and independent relays subserving different sensory modalities.

Ascending nociceptive pathways from the trigeminocervical complex

This discussion has been adapted from the following reviews: Craig and Dostrovsky, 1997; Craig and Dostrovsky, 1999; Dostrovsky and Strassman, 2000.

Nociceptive stimulation activates neurons in multiple ascending pathways projecting to various brainstem, thalamic, hypothalamic and telencephalic sites. Together these parallel systems distribute sensory information to multiple cortical regions. They process and integrate discriminative information and generate affective, homeostatic and motor responses appropriate to the behavioural state of the animal. Ascending projections may be monosynaptic direct projections, or indirect, involving polysynaptic pathways. The principal ascending pathways responsible for conveying nociceptive information are:

- a) The trigemino/quinto-thalamic tract with direct projections to thalamic nuclei.
- b) The projections to bulbar and mesencephalic nuclei which are analogous to the spinoreticular and spinomesencephalic tracts. These serve to integrate the responses of homeostatic control centres and also monoaminergic modulatory centres. These influence arousal and also have modulatory actions, both ascending and descending, on nociceptive neurotransmission.
- c) The projections to the hypothalamus.

Indirect ascending systems are also present such as a prominent bilateral projection to the parabrachial and Kölliker-Fuse nuclei. Many of the neurons activated by somatosensory stimulation in these areas respond to nociceptive stimuli. This projection may form a trigemino-ponto-amygdaloid pathway that is not relayed in the thalamus (Bernard et al., 1989). This pathway could have an important function in generating the behavioural, affective and autonomic reactions to head pain.

Trigeminothalamic Projections

The trigeminothalamic tract is the projection most closely involved in trigeminal nociceptive and thermal sensation. All of the trigeminal subnuclei send nociceptive afferents to the thalamus. The majority of these projections ascend to the contralateral thalamus, but in the cat and monkey a significant ipsilateral projection is also observed.

Numerous studies using axonal degeneration techniques and injection of retro- and anterograde anatomical tracers, such as horseradish peroxidase and wheat germ agglutinin, have demonstrated that the cells of the trigeminothalamic tract originate mainly from three regions of the Vc/medullary dorsal horn grey matter.

- 1) Lamina I and II_o, or the marginal zone and the outer zone of the substantia gelatinosa (mostly NS neurons).
- 2) Laminae IV to V, corresponding to the lateral part of the subnucleus reticularis dorsalis (WDR and LT neurons).
- 3) Laminae VII to VIII - the intermediate zone - which in Vc equates to the subnucleus reticularis ventralis (Shigenaga et al., 1983). These are complex cells, often with large receptive fields and are responsive to innocuous or noxious cutaneous stimulation.

This functional segregation of specific neurons into these laminae is however not absolute. In the spinothalamic tract approximately 50% of cells arise from lamina I with the remainder composed equally of cells from the two other regions. It is reasonable to assume that these same relative proportions apply in the trigeminothalamic tract. The trigeminothalamic tract merges with the spinothalamic (STT) tract at the level of the spinomedullary junction. It joins the medial aspect of the STT and the unified tract shifts dorsally, ascending through the midbrain to the diencephalon. The STT fibres are then distributed to multiple thalamic nuclei.

The Thalamus

Embryological development of the thalamus

The following discussion has been adapted from Sherman and Guillery (2001) and Steriade, Jones and McCormick (1997).

During its embryological development the diencephalon is divided into 4 regions: the dorsal thalamus, ventral thalamus, epithalamus and hypothalamus. The dorsal thalamus is the largest part of the mammalian diencephalon (it is commonly simply

referred to as the thalamus). Its size is intrinsically linked to the development of the cerebral cortex. The dorsal thalamus differs from the rest of the diencephalon in that it makes nearly all of its efferent connections with telencephalic structures, predominantly the neocortex. The ventral thalamus lies between the dorsal thalamus and the telencephalon during the diencephalon's embryological development. As a result it is perforated by axonal connections which form between the cortex and dorsal thalamus. The ventral thalamus sends no axons to the cortex in the mature brain. In the developed brain the ventral thalamus forms the thalamic reticular nucleus, a sheet-like nucleus of GABAergic neurons which is closely related to the thalamus. The epithalamus develops into the pineal body, habenular nuclei and a few other smaller dorsal nuclei.

Anatomy of the dorsal thalamus

The thalamus is a complex structure, composed of functionally and histologically distinct nuclei which form the lateral walls of the third ventricle (Fig. 3a). The Y-shaped internal medullary lamina (containing neurons arranged to form the sheet-like intralaminar nuclei) divides the thalamus into anterior, medial and lateral areas. Within the lateral area the nuclei are arranged into two tiers: ventral and dorsal. More detailed examination reveals that three distinct nuclear groups form the ventral tier, these are (from anterior to posterior): the ventral anterior, ventral lateral and the ventral posterior. The ventral posterior nuclear group contains the main somatosensory relay nuclei. In the monkey the ventral posterior nuclear group can be divided into the ventral posterior medial (VPM), ventral posterior medial parvocellular (VPMpc), ventral posterior lateral (VPL) and the ventral posterior inferior (VPI) (though this is not present in rats) nuclei. The term ventrobasal complex (VB) is used to denote those parts of the ventral posterior nucleus that convey sensory information to the SI and SII cortex. In practice in the rat the VB complex is synonymous with the VPM and VPL (Yokota, 1989). An additional important structural difference between rats and higher mammals is the relative absence of intrinsic interneurons within the VB complex. The ventrobasal thalamus of the rat contains only a few local circuit interneurons (Barbaresi et al., 1986). In higher mammals they constitute between 20 – 30% of the total cell count but only approximately 1% in the rat. These local circuit

interneurons contribute to the triadic synaptic arrangements observed in thalamic glomeruli. They participate in feed-forward inhibitory circuits, contributing to the modulation of afferent sensory inputs destined for the cortex.

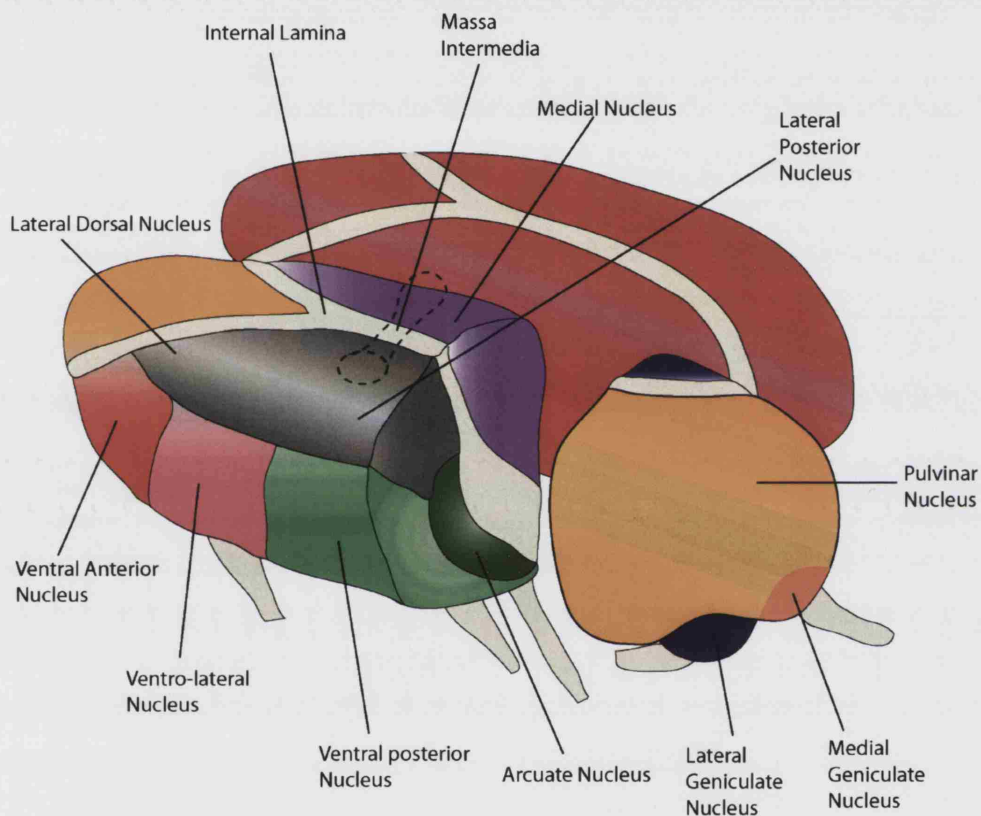


Figure 4a: Anatomy of the dorsal thalamus and connections

The dorsal thalamus is a complex structure which is divided into three by the Y-shaped internal medullary lamina. The ventral posterior nucleus is the main relay for sensory tracts conveying discriminative sensation to the primary (SI) somatosensory cortex. The VPM nucleus is located in its medial aspect and this conveys sensory information from the cranio-facial region. Adapted from: <http://mind-brain.com/thalamus.php>.

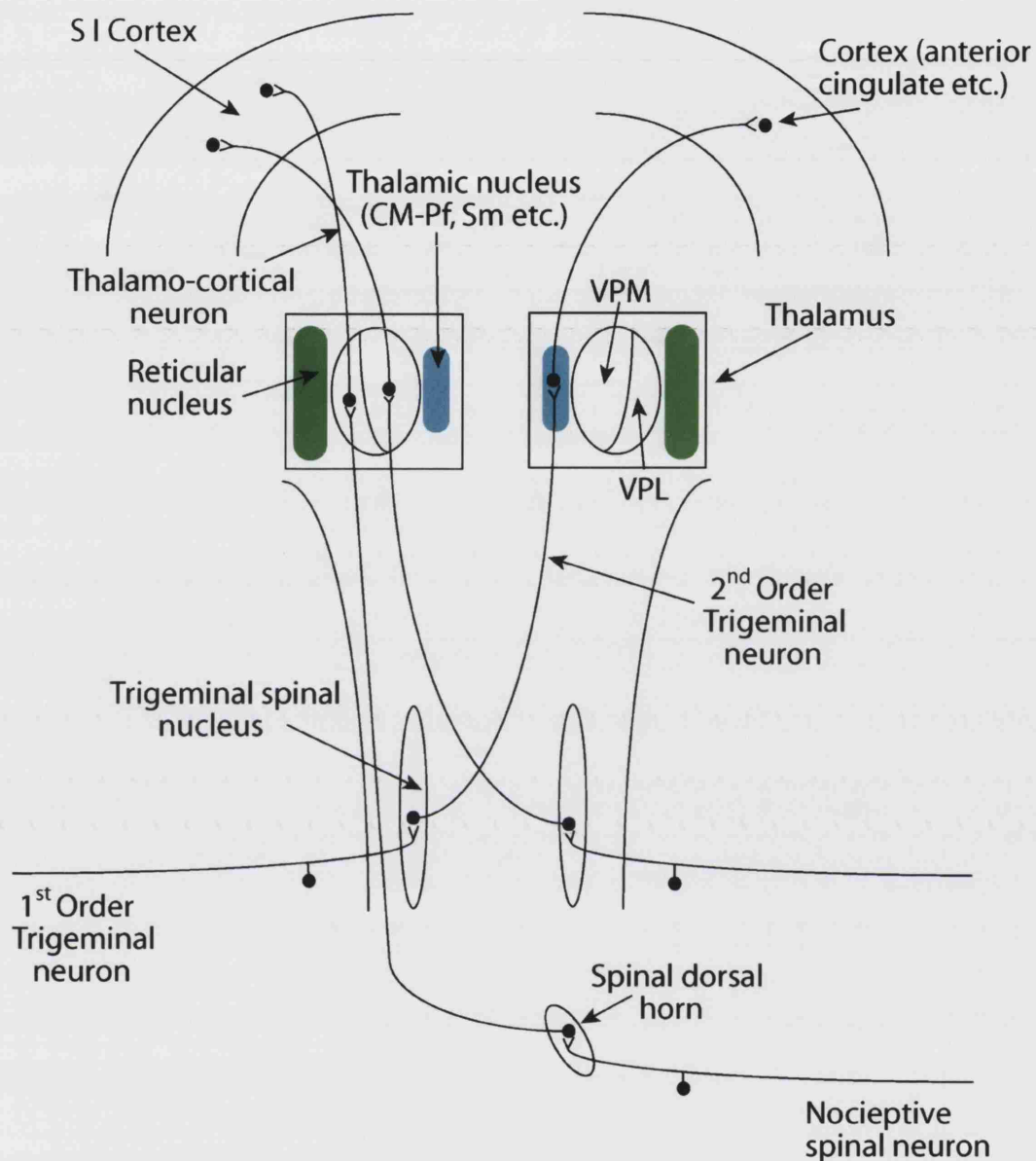


Figure 5b: Anatomy of the dorsal thalamus and connections

First order trigeminal neurons conveying nociceptive information relay in the spinal trigeminal nucleus and second order cells project via the trigemino/quintothalamic tract to the VPM nucleus of the thalamus. Nociceptive information from the rest of the body is conveyed via the spinothalamic tract to the more laterally placed VPL nucleus. Several parallel ascending pathways are present as trigeminal neurons conveying nociceptive information also project to other thalamic nuclei including the submedial nucleus (Sm), zona incerta and the central median-parafascicular complex (CM-Pf). These nuclei project to various cortical regions including the anterior cingulate and inferior orbital cortices. This is unlike VPM and VPL which project to the primary somatosensory (S I) cortex. (CM-Pf: central median-parafascicular complex; S I: primary somatosensory cortex; Sm: submedial nucleus; VPL: ventroposterolateral nucleus of the thalamus; VPM: ventroposteromedial nucleus of the thalamus)

The VPM nucleus of the thalamus as a relay centre for nociceptive information

Trigeminothalamic and spinothalamic projections terminate in several thalamic nuclei (Fig. 3b). While each of these appear to be involved in the parallel processing of nociceptive information, the function of the VB complex is the best understood. In the following discussion the experimental evidence obtained from studies of animal and human studies shall be reviewed.

Experimental studies of nociceptive neurotransmission in the rat VPM

In the rat anatomical studies using degenerative techniques (Lund and Webster, 1967) and injection of axonal tracers (Cliffer et al., 1991; Iwata et al., 1992; Kemplay and Webster, 1989; Peschanski, 1984) demonstrate that afferents from Vc project to the contralateral VPM. The VB complex is arranged somatotopically with craniofacial structures represented medially in the VPM while the rest of the body is found laterally in the VPL (Angel and Clarke, 1975; Emmers, 1965; Waite, 1973b). Within the VPM neurons with V_I receptive fields are found dorsally, while V_{III} responsive neurons are found in its ventral aspect (Shigenaga et al., 1973). The terminations of lemniscal (Feldman and Kruger, 1980) and spino/trigeminothalamic tracts overlap throughout the VB complex (Ma et al., 1986). As a result inputs conveyed in separate somatosensory pathways, but with similar receptive fields, tend to converge in the same region of the VPM. When examined at the ultrastructural level spinothalamic and lemniscal terminals have large “bushy arbours” (Peschanski, 1984). These terminal profiles form synaptic contacts on over-lapping regions of the (proximal) dendritic trees of thalamocortical neurons (Ma et al., 1987; Peschanski, 1984; Williams et al., 1994). These large terminal arbours are thought to ensure that afferent information is transmitted with a high degree of reliability through apparent redundancy.

Electrophysiological studies of nociception in the rat VPM

While many VB neurons respond to tactile stimulation of the skin or vibrissae (Berkley et al., 1993; Cropper and Eisenman, 1986; Raboisson et al., 1989) nociceptive (both NS and WDR) neurons, are also frequently found (Angel and Clarke, 1975; Cropper and Eisenman, 1986; Guilbaud et al., 1981; Guilbaud et al.,

1980; Peschanski et al., 1980; Yen et al., 1989). Somatic nociceptive stimulation results in the expression of *c-fos* IR within the VB complex (Bullitt, 1989). Tracing studies employing anterograde transport of Herpes simplex virus from the tooth pulp, which is assumed to only contain nociceptive neurons, confirm that the VPM forms part of the pathway from the periphery to the somatosensory cortex (Barnett et al., 1995). A higher proportion of nociceptive responsive VPM neurons appear to respond to purely nociceptive (NS) stimulation of their peripheral receptive fields than WDR stimulation (Peschanski et al., 1980). Unfortunately electrophysiological studies of the thalamus are complicated by the effects of anaesthetic agents. Differences in anaesthetic level may account for many of the discrepancies between studies. Anaesthetic agents have profound effects on the response properties of nociceptive VB neurons. As the depth of anaesthesia increases the receptive field properties of neurons may be substantially modified. Neurons which were characterised as nociceptive subsequently only respond to innocuous stimulation, often with larger receptive fields (Guilbaud et al., 1981).

The response properties of VB complex neurons in the rat indicate that they are capable of conveying the sensory discriminative aspects of nociception. Their receptive fields are often small, similar to LT neurons (Raboisson et al., 1989; Yen et al., 1989), and therefore suitable for stimulus localisation. Some studies however have described neurons responding to nociceptive stimulation from large areas of the body - which may even extend across the midline (Cropper and Eisenman, 1986; Guilbaud et al., 1980). Noxious heat is also encoded by VB nociceptive neurons. Thermal thresholds for VB neurons are the same as those that activate dorsal horn neurons and elicit behavioural withdrawal responses (Mitchell and Hellon, 1977). The response of neurons increases in proportion to the intensity and extent of the exposure (Mitchell and Hellon, 1977; Peschanski et al., 1980). This monotonic relationship between stimulus intensity and discharge frequency closely mirrors findings in humans, where the level of pain experienced is proportionate to the rate of action potential firing in response to noxious stimulation (Gybels et al., 1979; LaMotte and Campbell, 1978).

Nociceptive viscerosomatic convergence in the rat VPM

A large proportion of VB neurons are also activated by visceral nociceptive stimulation (Al-Chaer et al., 1996; Berkley et al., 1993; Guilbaud et al., 1993; Zhang

et al., 2002; Zhang et al., 2003). These neurons are not viscerotopically arranged in the thalamus (Berkley et al., 1993), though they do exhibit viscerosomatic convergence. While a small number of neurons are only responsive to visceral stimulation, the majority have cutaneous receptive fields which can be characterised as either nociceptive or responsive to innocuous stimulation. Interestingly visceral nociceptive information appears to be carried in the dorsal column pathway and not in the STT as might have been expected (Al-Chaer et al., 1996) and there is some evidence of convergence of spinothalamic and lemniscal inputs at the thalamic level (Ma et al., 1986; Ma et al., 1987; Peschanski, 1984). Thalamic responses elicited by noxious visceral inputs differ from those produced by somatic nociceptive stimuli. They tend to be delayed in onset and have long after discharges in comparison to somatic evoked responses (Berkley et al., 1993). Visceral and somatic inputs appear to be able to mutually modulate their respective responses (Zhang et al., 2002; Zhang et al., 2003). Conditioning visceral nociceptive stimuli can enhance the responses of VB neurons to somatic nociceptive activation (Zhang et al., 2003). This may explain in part the referred allodynia often observed in association with visceral pain (Burstein et al., 2000a; Burstein et al., 2000b).

Vibrissal inputs to the VPM

In rats the vibrissae serve an important sensory function for exploring the environment. As expected there is a strict somatotopic organisation with different horizontal lines of vibrissae represented at different rostro-caudal levels within the VPM (Waite, 1973b). The number of vibrissae in the receptive field of an individual VPM neuron ranges from 1 up to as many as 8, though even then there is typically a central whisker that triggers a maximum response (Chiaia et al., 1991a; Chiaia et al., 1991b; Diamond et al., 1992; Waite, 1973a). The thalamic processing of vibrissal-like nociceptive information also appears to be dependent on the type and depth of anaesthesia (Friedberg et al., 1999) with a one-to-one relationship between vibrissae and VPM neurons more likely at deeper anaesthetic levels (Guedel stage III₄). Cytochrome oxidase staining of the primate VPM reveals a darkly staining rod and a lighter matrix domain each receiving separate inputs from different regions of the trigeminal nucleus (see below). Cytochrome oxidase staining of the rat VPM also reveals a similar segregation which is likely to have a functional significance like the

rods and matrix of the primate somatosensory thalamus. In the rat VPM nucleus these rod-like structures are called barreloids. Barreloids are aggregates of neurons (van der Loos, 1976) and each neuron in a given barreloid responds to movement of the same whisker. They receive their inputs predominantly from Vp (Williams et al., 1994). Sensory deafferentation, such as following chronic trimming of the whiskers, results in a loss of this differential staining pattern in barreloids presumably due to underlying metabolic changes (Land and Akhtar, 1987). In contrast the spinal trigeminal nucleus sends afferents only to the outer fringes of individual barreloids, the inter-barreloid septae and the caudal portion of VPM, an area largely devoid of barreloids.

This is however as far as the parallels between rat and primate thalamus may be taken. Most obviously the rat VPM has a much larger volume dedicated to the vibrissal representation (Vahle-Hinz and Gottschaldt, 1983) than either the cat or monkey. Unlike primates there is also a homogeneous pattern of calcium binding protein immunoreactivity throughout the VPM (Williams et al., 1994), though the significance of this is not known.

Experimental studies of nociceptive neurotransmission in the cat VPM

The transmission of nociceptive information has been extensively studied in the felid VB complex. As in the rat, the effects of anaesthesia have lead to conflicting reports on the presence and nature of nociceptive responsive neurons (Haimann et al., 1978). The VPM in the cat receives afferent projections from each of the trigeminal subnuclei; in particular Vp and caudal Vi (Burton and Craig, 1979; Matsushita et al., 1982). Nociceptive projections to the VB complex come mainly from the trigeminocervical complex. Cooling of the contralateral surface of the medulla oblongata temporarily abolishes the response to nociceptive stimulation of a NS neuron's receptive field (Yokota et al., 1986). This is rendered permanent, while crucially having no effect on LTM neurons in the core of VPM, by performing a trigeminal tractotomy at the level of the obex (Yokota et al., 1986). The projections from Vc arise mainly from laminae I and V (Burton and Craig, 1979; Craig and Dostrovsky, 2001; Matsushita et al., 1982; Shigenaga et al., 1983). Though receiving a predominantly contralateral input from Vc, the cat VB differs from the rat in having a small ipsilateral projection as well.

The core of the VB complex contains somatotopically arranged LTM neurons while NS and WDR neurons are concentrated in the outer margin or “shell” region of the complex (Honda et al., 1983; Kniffki and Mizumura, 1983; Martin et al., 1990; Perl and Whitlock, 1961; Yokota et al., 1985; Yokota and Matsumoto, 1983; Yokota et al., 1986). This shell only appears to envelope the caudal third of the VPM. NS neurons in the shell have generally small, circumscribed receptive fields. In the actual NS zone, cells are somatotopically arranged such that the V_I representation is found in the dorsolateral shell, V_{II} dorsomedially and V_{III} in the ventromedial region. Nociceptive specific neurons are not found along the border between VPM and VPL, though they were found along the border between VPM and VPMpc. From the shell region thalamocortical neurons project to the SI cortex (Yokota et al., 1986). Rostral to this zone WDR neurons are found in a discrete band that measures approximately 300 μm in length (Yokota et al., 1986). This band displays the same somatotopic organisation as the NS zone. The receptive fields of WDR, like NS neurons, are small, of similar size to LTM cells and thus ideally suited for a sensory discriminative role (Honda et al., 1983; Kniffki and Mizumura, 1983; Martin et al., 1990; Perl and Whitlock, 1961; Yokota et al., 1985; Yokota and Matsumoto, 1983; Yokota et al., 1986). Thermoresponsive nociceptive neurons in the cat display similar responses to those in rats, as the magnitude of the neuronal response appears to be proportionate to the intensity of the thermal stimulus (Martin et al., 1990).

Viscerosomatic convergence

Neurons in the shell region are commonly activated by noxious visceral stimulation of most of the internal organs, such as pelvic, abdominal, thoracic and dental (Asato and Yokota, 1989; Bruggemann et al., 1994b; Horie and Yokota, 1990; Yokota et al., 1986). The vast majority of viscerally activated VB neurons exhibit viscerosomatic convergence. The cutaneous receptive fields of these cells are most often of the NS or WDR type (Asato and Yokota, 1989; Bruggemann et al., 1994b; Horie and Yokota, 1990; Yokota et al., 1986), though LTM have also been reported (Taguchi et al., 1987). They are also generally found in the dermatomes corresponding to the segments of the spinal cord from which the visceral nerves arise.

Experimental studies of nociceptive neurotransmission in the primate VPM

A large contralateral projection to the VPM, originating from the trigeminal nucleus caudalis, is observed in several primate species (Ganchrow, 1978; Tiwari and King, 1974). The cells of origin are both NS and WDR and their laminar distribution within Vc is the same as the cat and rat (Price et al., 1976; Willis et al., 1979). As with the cat there is also an ipsilateral projection from Vp to the medial aspect of VPM (Jones et al., 1986). The majority of STT terminals are found in the VB complex (VPL and VPI account for more than 50% of terminal labelling following injection of HRP into the STT (Gingold et al., 1991), though they are not uniformly distributed throughout the VB complex. Clustering of terminals has been reported in the VPL (Boivie, 1979) though the segregation is not as marked as in cats. This clustering arises from the differential termination of lemniscal and trigeminothalamic terminals in the rod and matrix domains of the VPM respectively (Rausell and Jones, 1991a). The former consists of elongated rods of large cells, immunoreactive for parvalbumin, that often run through the whole length of the VPM. The rod domain is innervated by afferents arising from Vp. The matrix domain envelops the rods but this surrounding layer is thicker in the dorsomedial, ventral and caudal poles of VPM (Rausell and Jones, 1991b). The matrix is composed of smaller, calbindin immunoreactive cells that receive inputs from Vc. As in the other species reviewed, VB neurons project to the SI cortex (Chandler et al., 1992; Kenshalo et al., 1980; Rausell and Jones, 1991a). In the case of the rods they project selectively to the deeper layers of the SI cortex, while the matrix projects to layer I.

Electrophysiological characteristics of nociceptive responsive neurons

Approximately 10-20% of VPM neurons respond to nociceptive stimulation of their receptive fields (Bushnell and Duncan, 1987; Bushnell et al., 1993), consistent with the rest of the VB complex (Apkarian and Shi, 1994; Morrow and Casey, 1992) though a frequency of up to 50% has been reported (Chung et al., 1986). Anaesthesia does not appear to have a significant influence on this proportion. More nociceptive responsive cells are of the WDR variety than NS and many are activated by noxious heating (Bushnell et al., 1993; Chung et al., 1986; Kenshalo et al., 1980; Morrow and Casey, 1992). They also tend to be more common in the caudal, “matrix rich” third of VPM (Bushnell and Duncan, 1987). These neurons have small, contralateral, well

defined receptive fields which are often smaller than the receptive fields of dorsal horn neurons (Bushnell and Duncan, 1987; Kenshalo et al., 1980). In the face they are typically confined to the territory of a single division of the trigeminal nerve (Bushnell and Duncan, 1987; Bushnell et al., 1993). Thermoreceptive cells responsive to noxious heat are capable of discriminating small temperature changes with a high degree of accuracy (Bushnell et al., 1993). Temporarily inactivating VPM neurons reversibly inhibits trained behavioural responses to thermal stimulation of the face, indicating that VPM neurons are necessary for the perception of noxious thermal stimulation (Duncan et al., 1993).

Viscerosomatic convergence in the primate VPM

Most VPL neurons in anaesthetised primates respond to both visceral and somatic stimulation. Often neurons may be activated by stimulation of several visceral organs indicating viscerovisceral in addition to viscerosomatic convergence (Bruggemann et al., 1994a). There does not appear to be a visceral somatotopic organisation and there appears to be no consistent link between a neuron's somatic receptive field and its convergent visceral input (Bruggemann et al., 1994a; Chandler et al., 1992). Viscerosomatic thalamic neurons tend to have cutaneous receptive fields which respond to innocuous touch though NS cells have also been reported (Bruggemann et al., 1994a; Chandler et al., 1992).

Experimental studies of nociceptive neurotransmission in the human VPM

The VP nucleus in humans, also called the ventrocaudal nucleus, appears to be organised in a similar somatotopic fashion as the primate ventrobasal complex (Lenz et al., 1988). Cells activated by mechanical and thermal nociceptive stimulation of cutaneous receptive fields have been found in the core, as generally WDR neurons and posterior inferior regions of Vc, as NS neurons (Lenz et al., 1993a; Lenz et al., 1993b). As in primates, nociceptive cells appear to be clustered together. Pain may be elicited by electrical stimulation of areas containing nociceptive neurons, particularly in the posterior inferior region where NS cells are found (Lenz et al., 1993b). Functional imaging studies in humans using PET show contralateral thalamic blood flow changes in response to nociceptive thermal stimulation, while visceral pain causes bilateral changes (Casey et al., 1994). Limitations in resolution however

preclude precise anatomical localisation - though these problems are beginning to be overcome with functional magnetic resonance imaging (fMRI). This technique demonstrates that thermal stimulation of trigeminal dermatomes activates the human equivalent of the VPM nucleus (DaSilva et al., 2002). As with the other species studied, the human thalamus also receives sensory inputs from visceral structures. Electrical stimulation may provoke unpleasant sensations of a visceral nature (Davis et al., 1995; Lenz et al., 1994). Interestingly electrical stimulation of the somatosensory thalamus (VPL) may trigger a visceral-type head pain that is almost clinically indistinguishable from migraine (Raskin et al., 1987).

Trigeminovascular nociceptive neurotransmission in the thalamus.

Only a very limited number of studies have examined this topic and all have been performed in the cat. SSS stimulation results in increased blood flow and metabolic activity (as measured by 2-deoxy-D-[1¹⁴C]-glucose uptake) in the thalamus (Goadsby et al., 1991). Metabolic activity increases substantially in the VPM but remains unchanged in the VPL. Lesser increases were also observed in POM. Antidromic activation of nociceptive neurons in the TCC from the VB complex provides indirect electrophysiological evidence that the VPM acts as a relay for trigeminal nociception (Hu et al., 1981; Strassman et al., 1986). Systematic studies of the lateral thalamus reveal that trigeminovascular nociceptive information is relayed in several thalamic nuclei. These include the VPM and its ventral periphery, POM, the zona incerta (ZI), intralaminar complex and ventrolateral nucleus (VL) (Zagami and Lambert, 1990). This population of neurons appears to receive convergent inputs from multiple sources including cutaneous, vascular and tooth pulp afferents (Angus-Leppan et al., 1995; Davis and Dostrovsky, 1988b; Zagami and Lambert, 1990). Their cutaneous receptive fields tend to be located on the head or face. They were generally small, being confined to one division of the trigeminal nerve, and responsive to innocuous and nociceptive stimulation. Topical application of capsaicin and bradykinin to the SSS and middle meningeal artery also result in increased firing of thalamic neurons (Davis and Dostrovsky, 1988a; Zagami and Lambert, 1990; Zagami and Lambert, 1991).

As can be seen the VPM nucleus, and the human ventrocaudal equivalent, serve the important function of relaying nociceptive information to the primary somatosensory cortex in all the species examined. Though the segregation of neurons within the VPM may differ between species, there is a remarkable conservation of function between rat, cat, primate and human. VPM neurons, unlike those in the midline thalamic nuclei convey information regarding the discriminative nature of somatic sensory stimulation (Sherman and Guillery, 2001; Steriade et al., 1997). These same VPM neurons also receive convergent inputs from visceral structures. Symptoms, almost indistinguishable from those of angina, migraine and abdominal pain, may be elicited by electrical stimulation of neurons in this region. Electrical stimulation of intracranial vascular structures, notably the superior sagittal sinus, activates neurons in the VPM of the cat. It is reasonable to assume that electrical stimulation of the SSS will activate a similar population of neurons in the VPM of the rat (though in this species SSS responsive neurons would be expected to be found throughout the VPM nucleus). These neurons may be functionally similar to those conveying nociception following electrical stimulation of the human SSS. They may also be important in humans in the perception of pain in migraine.

Nociceptive neurotransmission in other thalamic nuclei

The following discussion has been adapted from Craig and Dostrovsky (1997) and Steriade et al. (1997). Several thalamic nuclei are involved in the parallel relaying and processing of somatosensory information. Nociceptive information is relayed to several cortical regions and the complex interactions of thalamocortical, corticothalamic and corticocortical circuits results in the perception of pain (Sherman and Guillery, 2001). The lateral thalamic nuclei project to the SI and SII cortices. These are responsible for the perception of the sensory discriminative aspects of pain. Medial nuclei however project largely to areas of the cortex thought to be involved in the motivational-affective components of pain. These include the orbital cortex, anterior cingulate gyrus and by way of the insula, to the limbic system. *C-fos* IR immunoreactivity can be detected in several thalamic nuclei in the rat following nociceptive stimulation. This was particularly marked in the midline nuclei including: central lateral, paracentral, central median, parafascicular, reunions and the paraventricular, where labelling was bilateral. Contralateral labelling was observed in

the submedius and zona incerta in addition to the expected labelling in the VB complex (Bullitt, 1989). This does not represent all of the nuclei thought to be involved in the relay of nociceptive information and it indicates the complexity of nociceptive processing. Though less is known about the function of these nuclei in comparison to the nuclei of the VB complex, some general observations may be made:

a) The posterior nuclei (Po) appear to have a role in the transmission of innocuous and noxious tactile information (Curry and Gordon, 1972). The neurons of Po appear to be very sensitive to the effects of anaesthesia (Curry and Gordon, 1972). This may account for the large differences in the proportions of neurons responsive to nociceptive versus innocuous stimulation observed between studies. WDR and NS neurons are present in roughly equal numbers and their receptive fields have been described as both large and bilateral, or small and contralateral (Apkarian and Shi, 1994).

b) The intralaminar nuclei, generally taken to mean the central median-parafascicular complex (CM-Pf) may also be activated by nociceptive stimulation (Albe-Fessard and Kruger, 1962; Bushnell and Duncan, 1989; Dong et al., 1978; Peschanski et al., 1981; Reyes-Vazquez et al., 1986; Reyes-Vazquez et al., 1989; Urabe et al., 1966), though this is also sensitive to the type and depth of anaesthesia (Dong et al., 1978). Nociceptive responses are rare under deep barbiturate or chloralose anaesthesia. Again nociceptive cells may be classified as either NS or WDR and are capable of discriminating the intensity of nociceptive stimulation (Dong et al., 1978). Their receptive fields however tend to be large, bilateral and receive convergent inputs from both superficial and deep structures (Bushnell and Duncan, 1989; Dong et al., 1978; Peschanski et al., 1981; Urabe et al., 1966). Within the complex there appears to be no somatotopic organisation implying that these cells have no role in stimulus localisation (Nyquist and Greenhoot, 1974).

c) The submedius nucleus (Sm) is particularly well defined in rats, though it is also present in cats and primates. This is thought to be an important nociceptive relay nucleus (Craig and Burton, 1981; Dostrovsky and Guilbaud, 1988). The submedius nucleus conveys sensory information to the ventrolateral orbital cortex (Yoshida et al.,

1992). In rats this nucleus receives a large trigeminal projection which also contains thermoreceptive cells. Spinal cord projections are however much smaller (Dado and Giesler, 1990). The receptive fields, as with the other midline nuclei, tend to be larger than those seen with VB complex neurons. They are also often bilateral (Craig and Burton, 1981; Miletic and Coffield, 1989).

d) Recordings have been made from zona incerta (ZI) neurons responsive to deep somatic and nociceptive stimulation. ZI receives a laminated projection from the spinal cord. It is particularly large from the upper cervical segments (C₁-C₃), smaller from the lumbar spine and virtually non-existent from the thoracic cord. In the cervical region the projection arises from laminae IV-V and from lamina X in the lumbar spinal segments. This suggests that ZI cells are activated by deep somatic and visceral rather than cutaneous afferents (Shaw and Mitrofanis, 2001). The ZI is divided into dorsal and ventral regions. The receptive fields in the dorsal zone tend to be large, often covering the whole of the vibrissal pad and responsive only to light touch. In the ventral zone receptive fields are smaller, ranging from several to single vibrissae. Cells of all three modalities are found. Most of the ZI efferents are GABAergic, projecting to layer I of the SI cortex or subcortical structures such as the superior colliculus. It may therefore serve a modulatory function (Nicoletis et al., 1992).

e) The posterior part of the ventral medial nucleus (VMpo) appears to be a specific relay nucleus for nociceptive and thermoreceptive information in primates and humans. Like Sm, it receives afferents from lamina I but projects to the insula cortex. Neurons in VMpo are immunoreactive for calbindin, have small, somatotopically organised receptive fields and their discharge rates are graded in proportion to supra-threshold stimulus intensity (Craig et al., 1994).

Glutamate is the principal excitatory neurotransmitter in the sensory thalamus

Glutamate plays a crucial role in the transmission of nociceptive information in the VB complex. It is involved in signalling from STT and lemniscal pathways and corticothalamic afferents (Broman and Ottersen, 1992). Extracellular levels are increased following experimentally produced pain (Silva et al., 2001). It triggers post-

synaptic excitatory potentials (EPSPs) by activating multiple glutamate receptors. Both NMDA and non-NMDA glutamate type receptors (including metabotropic Group I receptors (mGlu1 and 5)) are involved in the transmission of sensory information (Dougherty et al., 1996; McCormick, 1992; Salt, 1986; Salt, 1987; Salt and Binns, 2000; Salt and Eaton, 1990).

Modulation of thalamocortical neurons

Thalamocortical neurons in the VPM receive modulatory inputs from several sources. These modulatory inputs significantly out-number sensory afferents. They have characteristics that distinguish them from sensory afferents (summarised in Table 3). In general modulatory inputs form relatively small synaptic contacts with thalamocortical cells. This is quite different to sensory afferents which have large terminal arbours and form extensive synaptic contacts. These are found in the region of the somata and proximal dendrites, whereas modulatory synaptic contacts may be found over the entire dendritic tree. Modulatory inputs activate metabotropic and ionotropic receptors (sensory afferents make use of only ionotropic receptors). Modulatory afferents are further distinguished by their inputs to the reticular nucleus (itself a modulatory input to VPM neurons) and possibly a high degree of convergence onto relay cells.

Sources of modulatory inputs (and major neurotransmitters) to the sensory thalamus include:

- a) cortex (glutamatergic)
- b) reticular nucleus (GABAergic)
- c) raphe nuclei (serotonergic)
- d) locus coeruleus (noradrenergic)
- e) pontine tegmental nuclei (cholinergic)
- f) hypothalamic (histaminergic)

Table 3: Comparison of the characteristics of sensory and modulatory thalamic afferents

Characteristic	Sensory afferents	Modulatory afferents
Terminal size	Large	Small
Reticular nucleus innervation	No	Yes
Activate ionotropic receptors	Yes	Yes
Activate metabotropic receptors	No	Yes
Afferent convergence	No	Possibly

Adapted from (Sherman and Guillery, 2001)

The Reticular nucleus and GABAergic modulation

GABA Receptors

GABA is the most widely distributed inhibitory neurotransmitter in the vertebrate CNS (Sivilotti and Nistri, 1991). At present three GABA receptors are recognised; the ionotropic GABA_A and GABA_C (also known as GABA_{A0r}) and the metabotropic GABA_B receptor. GABA_C receptors are a class of bicuculline- and baclofen-insensitive GABA receptor which were identified in the retina of vertebrates (Bormann, 2000).

GABA_A receptors are pentameric, ligand gated chloride (Cl⁻) channels (Barnard, 2001). Presently 18 mammalian subunits are recognised (this does not include the possibility of further splice variants) which can be grouped into 7 sub-families depending on their sequence homology (α 1-6, γ 1-3, β 1-4, δ , ϵ , ρ 1-2, θ) (Cooper et al., 2003). This diversity raises the possibility of a large number of GABA_A receptor isoforms composed of various combinations of sub-types. Five subunits co-assemble to form the receptor/ionophore. The GABA_A receptor has modulatory binding sites for benzodiazepines, barbiturates, ethanol and neurosteroids (Mohler, 2001). When activated there is a large influx of Cl⁻ ions (and HCO₃⁻). This reduces the membrane resistance which effectively holds or “shunts” the membrane potential towards the reversal potential for Cl⁻ ions (-70mV). This produces a short lasting but potent hyperpolarisation of the cell membrane.

GABA_B receptors are metabotropic heterodimers. Two receptor subunits, B1 and B2, are currently recognised but they must combine to form a functional GABA_B receptor. The gene encoding B1 can be alternately spliced to produce two products: B1a and B1b. GABA_B receptors are coupled to potassium and calcium channels via G-proteins and second messenger systems. Post-synaptic GABA_B receptors are negatively coupled to adenylate cyclase via G_{vo} proteins. This results in an increase in K⁺ conductance through inward rectifying potassium channels (Kir 3.0) hyperpolarising the cell to approximately -100mV. The change in membrane conductance is much less than after GABA_A receptor activation so the membrane potential is less firmly held. In comparison with GABA_A receptor mediated hyperpolarisation, GABA_B hyperpolarisation however has a longer time-course (Bowerly, 2001).

Reticular nucleus modulation of sensory information.

The VB complex of rodents, unlike that of higher mammals, is largely devoid of GABAergic interneurons (Barbaresi et al., 1986). The principal source of GABAergic modulatory inputs in rodents therefore arises from the reticular nucleus (RT). There are other sources of GABAergic inputs which originate in the basal forebrain and striatum, but these largely innervate the medial nuclei (Steriade et al., 1997).

The RT is a thin layer of cells on the dorsolateral and ventral aspects of the thalamus. It is substantially smaller than the VB complex, containing less than half its number of neurons. All of the cells in the RT are immunoreactive for the GABA synthetic enzyme glutamic acid decarboxylase (Houser et al., 1980) and also GABA (de Biasi et al., 1986). The reticular nucleus is divided functionally into regions innervating different thalamic nuclei (Jones, 1975). The area innervating the VB complex is somatotopically organised (Shosaku et al., 1984). As with the VB complex, the representation of the craniofacial region, especially that of the vibrissae, is particularly large. The receptive fields of RT neurons are generally quite similar to their counterparts in the VB complex, though less specific – particularly with regard to vibrissal direction selectivity (Shosaku, 1985; Yen et al., 1985).

RT neurons receive glutaminergic axon collaterals from VB thalamocortical neurons. As VB neurons outnumber RT neurons, there is a large degree of convergence (Shosaku, 1986). RT neurons in turn send axons to the region of the VB complex containing neurons with corresponding receptive fields (Fig. 4). Each innervates

several VB neurons (Peschanski et al., 1983). This circuit is responsible for the stimulus induced feed-back inhibition observed in VPM neurons.

The modulatory actions of RT neurons are based on both GABA_A and GABA_B receptor activation. Some of the brain's highest concentrations of GABA receptors are found in the VB complex (Bowery et al., 1987). Additionally a broad range of GABA receptor subunits are expressed in the VB complex, possibly indicating a heterogeneous population of receptor isoforms. The main mRNAs in the monkey thalamus encoding GABA_A receptor subunits are $\alpha 1$, $\alpha 5$, $\beta 2$, $\gamma 2$ while in the rodent $\alpha 5$ transcripts are rare and $\alpha 4$ and $\beta 2$ are the most abundant (Huntsman et al., 1996; Khrestchatisky et al., 1989; Wisden et al., 1992). GABA_{B1a}, _{1b} and _{B2} are also found in high concentrations in the VB complex (Charles et al., 2001). GABA_A receptors are located post-synaptically. GABA_B receptors however are found both pre- and post-synaptically and are not confined to the immediate synaptic region (Chu et al., 1990; Kulik et al., 2002). The functions of pre-synaptic GABA_B receptors are not clearly defined. They may tonically modulate the pre-synaptic release of not only GABA (Emri et al., 1996) but also excitatory neurotransmitters (Nyitrai et al., 1996). The anti-nociceptive properties of GABAergic compounds are well documented (Bartolini et al., 1980; Buckett, 1980; Sawynok and LaBella, 1982; Vaught et al., 1985). Microiontophoretic studies indicate that GABA modulates nociceptive transmission in several thalamic regions including the: medial, ventromedial and centrolateral nuclei (Olausson et al., 1994; Reyes-Vazquez and Dafny, 1983; Reyes-Vazquez et al., 1986). It also modulates the activity of VPM neurons in models of chronic pain (Yamashiro et al., 1994; Yamashiro et al., 1997).

Activation of RT neurons results in inhibition of spontaneous and evoked activity in VB complex neurons (Mushiake et al., 1984). Destruction of the RT has the expected effect of increasing the response probability of neurons following receptive field stimulation. In addition relay cells demonstrate a change in the firing mode in response to sustained sensory stimulation, changing from phasic to tonic mode, similar to trigeminal ganglion cells (Hartings and Simons, 2000; Lee et al., 1994a). The size of the receptive field also increases substantially. It is possible that activation of GABA_A and GABA_B receptors modulates different aspects of the sensory information flowing to the cortex (Crunelli and Leresche, 1991; Vahle-Hinz et al., 1994). GABA_A receptor activation probably modulates the strength of sensory activation. GABA_B receptor mediated slow inhibition may alternatively serve to

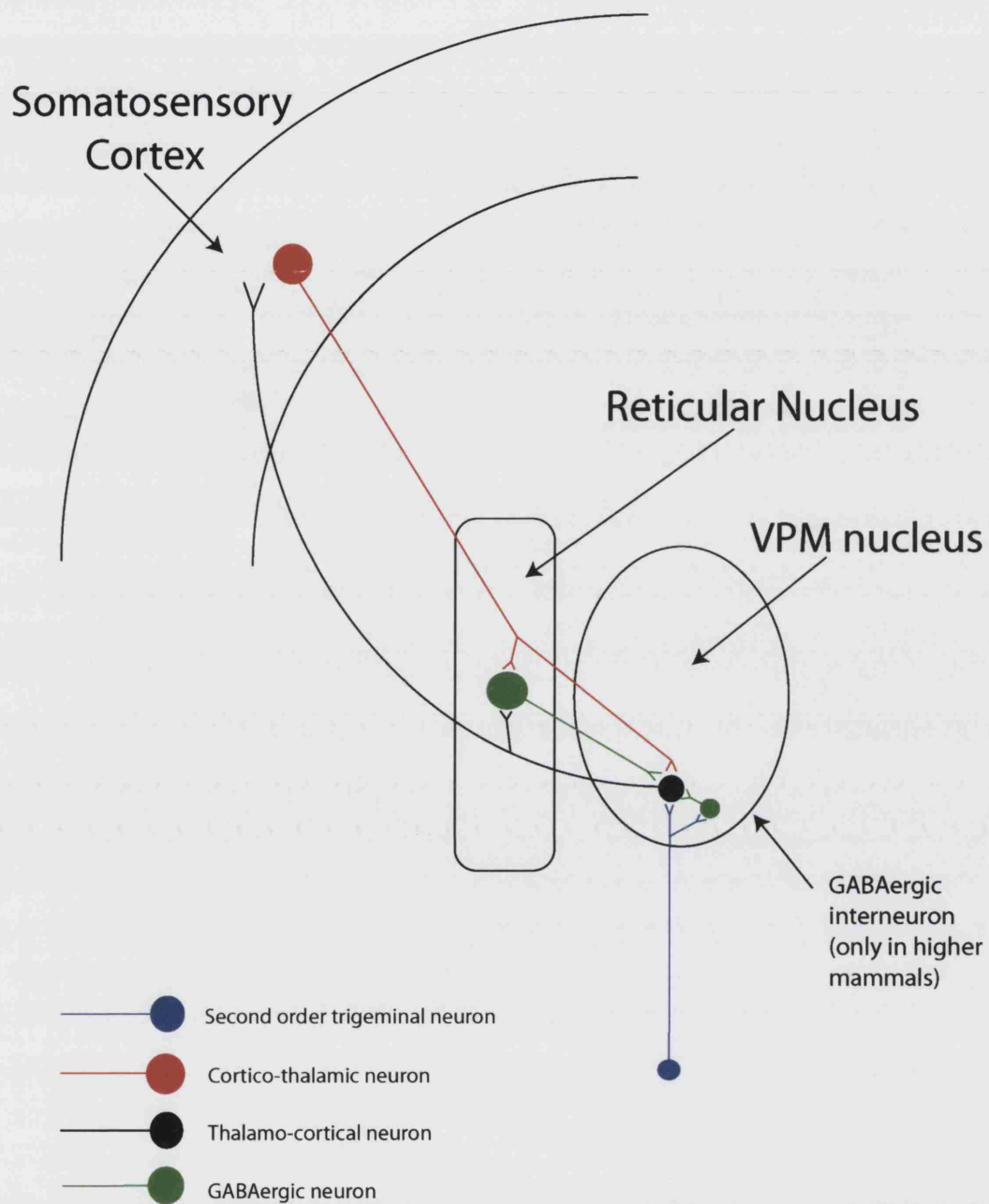


Figure 6: Connections between the VPM nucleus, SI cortex and reticular nucleus

GABAergic modulation of thalamocortical and corticothalamic circuits. Each thalamocortical neuron receives GABAergic inputs from inhibitory interneurons (feed-forward inhibition (not in rodents)) and also from neurons in the thalamic reticular nucleus. Reticular neurons are somatotopically organised, receiving (glutaminergic) collaterals from thalamocortical and corticothalamic neurons representing similar areas of the body. Reticular neurons provide feedback inhibition to thalamocortical neurons.

integrate inputs from surrounding sensory areas and also enhance temporal filtering (Lee et al., 1994b). The net effect may be to maintain the ability of cortical neurons to respond and differentiate transient sensory stimuli (Hartings and Simons, 2000).

Valproic Acid: mechanisms of action and role in migraine treatment

Valproic acid is a commonly prescribed migraine preventive (Silberstein, 1996). It is a simple branched chain carboxylic acid (2-propyl-pentanoic acid), but it has an unusually high degree of ionisation at neutral pH (Loscher, 1999). This renders it much less lipid soluble than most other anticonvulsants. Despite this it rapidly enters the brain, transported by a probenecid-sensitive carrier. It is extensively protein-bound and has a plasma half-life ($t_{1/2}$) of between 10-20 hours - plasma levels only stabilise roughly one week after dose changes. It also has active metabolites with long half-lives. Its efficacy as a prophylactic treatment for migraine has been confirmed by a number of trials (Hering and Kuritzky, 1992; Jensen et al., 1994; Klapper, 1997; Mathew et al., 1995). Migraine frequency may be cut by 50% for between 40-80% of patients. The intensity and duration of attacks may also be reduced. Plasma concentrations however do not correlate with anti-migraine efficacy, possibly resulting from nonlinear, concentration-dependent protein binding (Hering and Kuritzky, 1992; Jensen et al., 1994; Sorensen, 1988). Intravenous valproate has also been suggested as an acute rescue therapy for migraine from results of open label studies (Mathew et al., 2000; Stillman et al., 2004).

Valproic acid has a complex array of actions, though its main effect may be the facilitation of endogenous GABAergic neurotransmission (Johannessen, 2000; Loscher, 1999). There is substantial evidence that chronic treatment at clinically relevant doses increases concentrations of CNS GABA. This is particularly marked in the synaptosomal fractions of brain preparations, principally indicating increases in nerve terminals (Loscher, 1981; Loscher and Vetter, 1984; Loscher and Vetter, 1985). These increases show a regional variability, being most pronounced in areas such as the frontal cortex, hippocampus and hypothalamus with only a non-significant GABA concentration increase in the thalamus. How valproate brings about this increase in "pre-synaptic" GABA is a matter of considerable debate. Two main mechanisms are possible: a) inhibition of GABA degradation (inhibiting GABA transaminase or

succinate semialdehyde dehydrogenase), or b) enhancement of GABA synthesis (activating glutamic acid decarboxylase) (Johannessen, 2000; Loscher, 1999). Doubts however continue as to whether either actually occurs at physiological doses. Increases in synaptosomal concentrations may be detected within five minutes of administration of valproate, paralleling the onset of anti-nociceptive behaviour (Loscher and Vetter, 1985).

This increase in pre-synaptic GABA levels would only be able to augment GABAergic neurotransmission if there was some mechanism to also promote its release. Valproate increases GABA release in cultured neurons (Gram et al., 1988) and cortical slice preparations. Indirect evidence exists for this effect in humans as chronic treatment with valproate leads to increases in GABA concentration in the CSF (Loscher and Siemes, 1985; Zimmer et al., 1980). Though valproate appears to be able to directly augment the post-synaptic effects of GABA (Macdonald and Bergey, 1979) it probably only does so at very high doses, outside the physiological range.

Valproate has however a range of other actions, any of which could account for its anti-migraine properties (Loscher, 1999). Valproate administration may result in use dependant reductions of inward Na^+ currents (McLean and Macdonald, 1986). This has largely been inferred from studies where it inhibits sustained repetitive neuronal firing. It is assumed that in doing so valproate acts in a similar manner as phenytoin and carbamazepine. Valproate probably does not have a significant action on AMPA or kainate-type glutamate channels, but it may suppress NMDA evoked depolarisations (Zeise et al., 1991). Relatively low concentrations of valproate could inhibit this in rat neocortical slice preparations. This is potentially of relevance as NMDA-type glutamate channels are involved in sensory transmission within the ventrobasal complex (Salt and Eaton, 1991). Valproate has modulatory actions on two other neurotransmitters also implicated in the pathogenesis of migraine. Chronic administration leads to increases in levels of serotonin and dopamine in addition to their metabolites (Biggs et al., 1992; Whitton and Fowler, 1991; Zimmer et al., 1980). It is possible that the anti-migraine action of valproate is due to these actions and that its GABAergic properties, though important for its anticonvulsant action, are not relevant.

Valproate has potent anti-nociceptive properties (Guieu et al., 1993; Mesdjian et al., 1983). Extra-thalamic actions of valproate have been demonstrated in models of trigeminal activation. It was able to reduce *c-fos* IR expression in the dorsal horn

following chemical irritation of the dura mater by intracisternal capsaicin (Cutrer et al., 1995). It was also effective in reducing dural plasma protein extravasation following electrical stimulation of the trigeminal nerve (Lee et al., 1995). In both instances its action could be antagonised by the GABA_A antagonist bicuculline (Cutrer and Moskowitz, 1996). A central action in migraine is supported by a preliminary study examining its effects on cortical electrophysiological phenomena. Valproate treatment normalised cortical “excitability”, measured by phosphene threshold using TMS, in a small number of patients with migraine with aura. These patients also experienced a clinical improvement in their symptoms in this open labelled study (Mulleners et al., 2002).

The Rostral raphe nuclei and serotonergic modulation

Serotonin receptors

The following discussion has being adapted from Barnes and Sharp (1999), Baumgarten and Gothert (1997), Boess and Martin (1994) and Cooper et al. (2003). Fourteen serotonin receptors are currently recognised and these are divided into 7 sub-families (5-HT₁₋₇). The majority belong to the seven transmembrane spanning family of G-protein coupled metabotropic receptors, but the 5-HT₃ family of receptors differ in that they are all ligand-gated ion channels (Na⁺ and K⁺ ions).

There are 5 members of the 5-HT₁ subfamily (Table 4). All share the common feature of being negatively coupled to adenylate cyclase by G_{o/i} proteins. The reduction in cAMP levels results in neuronal hyperpolarisation brought about by increases in potassium ion conductance and in some cases a reduction in calcium conductance.

5-HT_{1A} receptors

The rat 5-HT_{1A} receptor has 89% sequence homology with the human receptor. Receptor concentrations are high in the hippocampus, cingulate and entorhinal cortices and the mesencephalic raphe nuclei, moderate in the thalamus and spinal trigeminal tract but low in the basal ganglia and cerebellum (Ito et al., 1999; Kia et al., 1996b; Pazos et al., 1987). 5-HT_{1A} receptors may be found at pre-, post- and extra-synaptic sites in association with serotonergic, cholinergic and glutaminergic neurons

(Chalmers and Watson, 1991; Kia et al., 1996a). Activation causes neuronal hyperpolarisation through opening of K^+ channels (Aghajanian and Andrade, 1997). 5-HT_{1A} receptors have high binding affinities for aryloxyalkylamine compounds, including β -blockers, such as propranolol and pindolol, but not atenolol (Nishio et al., 1989). This property is conferred by a single asparagine residue at position 385 (Guan et al., 1992). 5-HT_{1A} receptor agonists induce a fall in 5-HT release, and trigger the release of acetylcholine and noradrenaline in selected brain areas by an unknown mechanism. They also produce behavioural effects including hyperphagia, anxiolysis and increased sexual behaviour (Barnes and Sharp, 1999).

5-HT_{1B} and 5-HT_{1D} receptors in the CNS

Confusion has surrounded the nomenclature of 5-HT_{1B} and 5-HT_{1D} receptors but this has been helped by the identification of species homologues (Table 5) (Hartig et al., 1996). The rodent r5-HT_{1B} receptor profoundly differs from higher species homologues, including the human h5-HT_{1B} receptor, in drug binding properties. r5-HT_{1B} receptors have high binding affinities for drugs such as the aryloxyalkylamines, including certain β -blocking drugs such as propranolol, but not atenolol. In contrast they have reduced affinity for sumatriptan and 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) compared to h5-HT_{1B} receptors (Barnes and Sharp, 1999). Though the primary amino-acid sequences of the receptors have over 90% homology, a single amino-acid difference accounts for the pharmacological differences (h5-HT_{1B}: threonine 355, r5-HT_{1B}: asparagine 355) (Oksenberg et al., 1992; Parker et al., 1993). r5-HT_{1D} and h5-HT_{1D} receptors show similar drug binding properties. The h5-HT_{1B} and h5-HT_{1D} receptors show close primary sequence homology (77% within the 7 transmembrane domains) and their drug binding profiles are almost indistinguishable, indeed relatively selective ligands have only become available relatively recently. In the brain 5-HT_{1B} receptors outnumber 5-HT_{1D} receptors (Bruinvels et al., 1994a; Bruinvels et al., 1994b; Bruinvels et al., 1993; Varnas et al., 2001). 5-HT_{1B} receptors are found in the cortex, basal ganglia, hippocampus, cerebellum and cerebral arteries (Bruinvels et al., 1994a; Varnas et al., 2001). They may be located both pre- and post synaptically (Boschert et al., 1994; Voigt et al., 1991) and they may modulate neurotransmitter function in serotonergic and non-serotonergic neurons (Bruinvels et al., 1994a). 5-HT_{1D} receptors may have a more restricted distribution in the cortex,

trigeminal nucleus, the V ganglion and basal ganglia in particular though 5-HT_{1B} receptors may have been mistaken for 5-HT_{1D} receptors in the past (Bonaventure et al., 1998).

Table 4: Pharmacological characteristics of 5-HT₁ receptors

	5-HT₁ Receptors				
	5-HT_{1A}	5-HT_{1B}[#]	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}
Agonists	8-OH-DPAT	Sumatriptan	Sumatriptan	-	LY344864 Sumatriptan
Antagonists	S-WAY100135 Spiperone	SB-224289 GR127935	BRL-15572 GR127935*	-	-
Coupling mechanism	(-) AC	(-) AC	(-) AC	?	(-) AC
Membrane effects	Hyperpolarising via opening of K ⁺ channels	?	?	?	?
Receptor Distribution	Hippocampus, lateral septum, cingulate and entorhinal cortex, mesencephalic raphe nuclei	Basal ganglia (substantia nigra, globus pallidus, ventral pallidum)	Basal ganglia, hippocampus, cortex, PAG, dorsal raphe nucleus	Cortex, caudate nucleus, putamen [^]	Hippocampus, cortex (cingulate, entorhinal), dorsal raphe nucleus [§]

Few ligands show absolute specificity for individual 5-HT₁ receptors which has led to difficulties elucidating their distribution and function. The h5-HT_{1B} and h5-HT_{1D} receptors (see Table 5) have very similar pharmacological profiles, though relative selective ligands are now available e.g. BRL-15572 is reported to have a 60-fold greater affinity for 5-HT_{1D} than 5-HT_{1B} receptors. Though nearly all 5-HT₁ receptors appear to be negatively coupled to adenylate cyclase activity (AC), a mechanism of action on the cell membrane has only been demonstrated for 5-HT_{1A} receptors. [#]The pharmacological profiles of the r5HT_{1B} and h5-HT_{1B} receptors are distinctly different, notably in regard of their binding affinity for certain β -adrenoceptor antagonists (see text for details). *GR127935 has demonstrated activity as a partial agonist in some functional tests of recombinant 5-HT_{1D} receptors. [^]5-HT_{1E} receptor distribution has been inferred from non-5-HT_{1A/1B/1D/2C} [³H]-5-HT binding studies in human, mouse and guinea pig brain. [§]5-HT_{1F} receptor distribution determined by in situ hybridisation to detect mRNA. Adapted from (Barnes and Sharp, 1999).

5-HT_{1E} and 5-HT_{1F} receptors

Owing to the lack of selective ligands for 5-HT_{1E} receptors, less is known about their distribution and function. They show a similar affinity for serotonin as 5-HT_{1F} receptors. 5-HT_{1F} receptors differ however in regard to their higher binding affinity for sumatriptan (Barnes and Sharp, 1999). 5-HT_{1E} and 5-HT_{1F} receptor mRNA is found in the cortex, caudate, putamen and hypothalamus (Bruinvels et al., 1994a) with more 5-HT_{1F} receptors being possibly located in the thalamus and Vsp (Castro et al., 1997; Waeber and Moskowitz, 1995). 5-HT_{1F} ligands are of particular interest as they

have demonstrated an ability to modulate responses in models of peripheral trigeminal activation (Goadsby and Classey, 2003; Mitsikostas and Sanchez del Rio, 2001).

Table 5: Revised nomenclature for 5-HT_{1B} and 5-HT_{1D} receptors

Receptor	Species homologue	
	Human	Rat
5-HT _{1B}	h5-HT _{1B} (5HT _{1Dβ})	r5-HT _{1B}
5-HT _{1D}	h5-HT _{1D} (5HT _{1Dα})	r5-HT _{1D}

Previous receptor names are in parenthesis.
Adapted from (Hartig et al., 1996)

The rostral raphe nuclei

In the mammalian brain most serotonergic neurons have their cell bodies in or near the midline in the brainstem raphe nuclei (Fig. 5). A significant proportion – up to 20% of serotonergic neurons - is displaced into the adjacent reticular formation. The raphe nuclei may be divided into a rostral and caudal group of cells. The rostral group project to the forebrain while the caudal group send projections to the spinal cord, brainstem and cerebellum. Increasing neocortical and thalamic size in primates and humans is associated with development of the rostral raphe complex and non-raphe lateral 5-HT elements (Baumgarten and Grozdanovic, 1997). The dorsal raphe nucleus (DRN) is notable for specific structural and electrophysiological features. Serotonergic neurons have an abundance of dendro-dendritic, dendro-somatic and axodendritic synaptic contacts (Chazal and Ralston, 1987). This may account for some of the particular electrophysiological features of DRN cells and their sensitivity to autoreceptor (5-HT_{1A}) agonists. DRN neurons discharge at a slow (2-5 Hz) and regular rate while the animal is awake (Fornal and Jacobs, 1988). The discharge rate is strongly dependent on the behavioural state of the animal, ceasing during REM sleep. They are highly responsive to auditory and visual stimulation. A significant proportion of rostral raphe neurons however are not serotonergic (Baumgarten and Grozdanovic, 1997; Fornal and Jacobs, 1988).

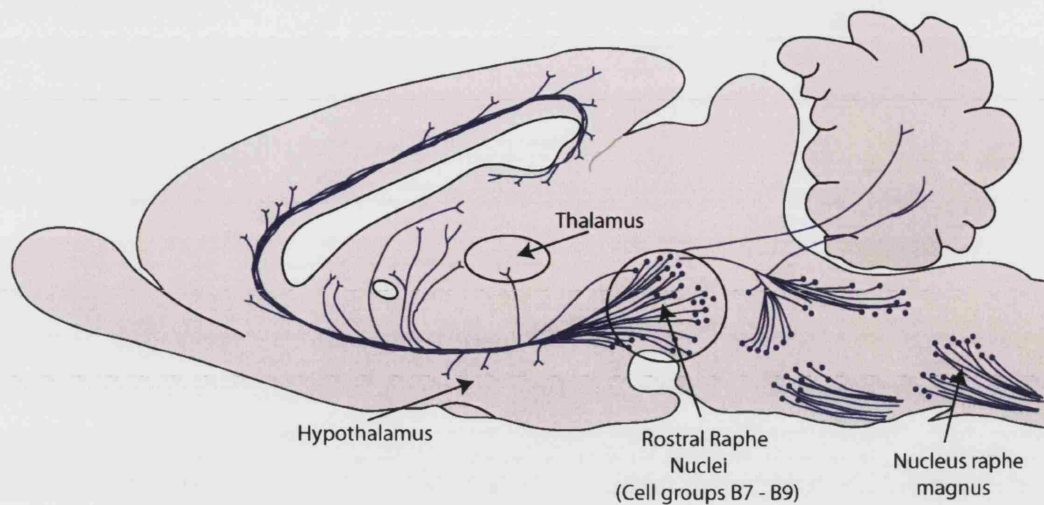


Figure 7: Central serotonergic projections

Coronal section through the rodent brain demonstrating central serotonergic projections. The rostral raphe nuclei (B7-B9; the dorsal raphe nucleus, medial raphe and the central superior raphe nucleus) project to the diencephalon (including the thalamus) and telencephalon. Caudal nuclei such as the nucleus raphe magnus (NRM) project to the brainstem and spinal cord. Adapted from (Kandel et al., 2000).

Serotonergic innervation of the VB complex

Biochemical studies have repeatedly demonstrated the presence of serotonin in the somatosensory VB complex of rats (Weil-Fugazza et al., 1986; Weil-Fugazza et al., 1988). Thalamic serotonin can be significantly reduced following lesioning of the median forebrain bundle (Anden et al., 1966). Demonstrating the serotonergic innervation histologically has proven however to be more difficult. Many early studies were only able to show 5-HT nerves in the mid-line thalamic nuclei (Parent et al., 1981; Vertes, 1991). Others could only show scant labelling in the ventral nuclei (Cropper et al., 1984; Fuxe, 1965; Steinbusch, 1981). More convincing evidence of a serotonergic innervation in the ventrobasal complex has been shown in a variety of species (Barbaresi et al., 1982; Moore et al., 1978; Schofield and Everitt, 1981; Westlund et al., 1990; Westlund et al., 1991), but the midline projection is still much larger. The 5-HT fibres are fine (perhaps explaining why they have been difficult to

demonstrate) with irregularly spaced varicosities forming loose networks in the vicinity of ventrobasal neuronal somata (Nothias et al., 1988). Serotonergic fibres project to the thalamus from the rostral raphe complex including the DRN, adjacent PAG (Chi, 1970; Consolazione et al., 1984) and the nucleus raphe medianus (Peschanski and Besson, 1984b).

5-HT₁ receptors in the VB complex

Problems with ligand selectivity and differences in the sensitivity of labelling techniques make it difficult to comment on the levels of various 5-HT₁ receptor subtypes in the VB complex. Non-selective autoradiographic mapping of 5-HT₁ receptors in both rodent and human brain reveal low levels of binding within the ventrobasal thalamus (Pazos and Palacios, 1985; Pazos et al., 1987).

In the rat thalamus 5-HT_{1A} receptor-like immunoreactivity is found in low levels but it is more pronounced in the midline nuclei (Aznar et al., 2003; Chalmers and Watson, 1991; Kia et al., 1996b). This labelling was found to be especially co-localised to calbindin positive cells, which correspond to projecting excitatory neurons (Frassoni et al., 1997).

Functional imaging studies in humans confirm the presence of 5-HT_{1A} receptors in the thalamus (Ito et al., 1999). Similarly 5-HT_{1B} and 5-HT_{1D} receptors and their mRNA were present in the thalamus of rats (Bruinvels et al., 1994a; Bruinvels et al., 1993; Langlois et al., 1995), guinea-pigs (Bonaventure et al., 1998) and humans (Varnas et al., 2001). Binding studies using [³H]sumatriptan demonstrate that a variety of 5-HT₁ receptors – including 5-HT_{1B/1D} and particularly 5-HT_{1F} - are found in the guinea pig thalamus (Waeber and Moskowitz, 1995).

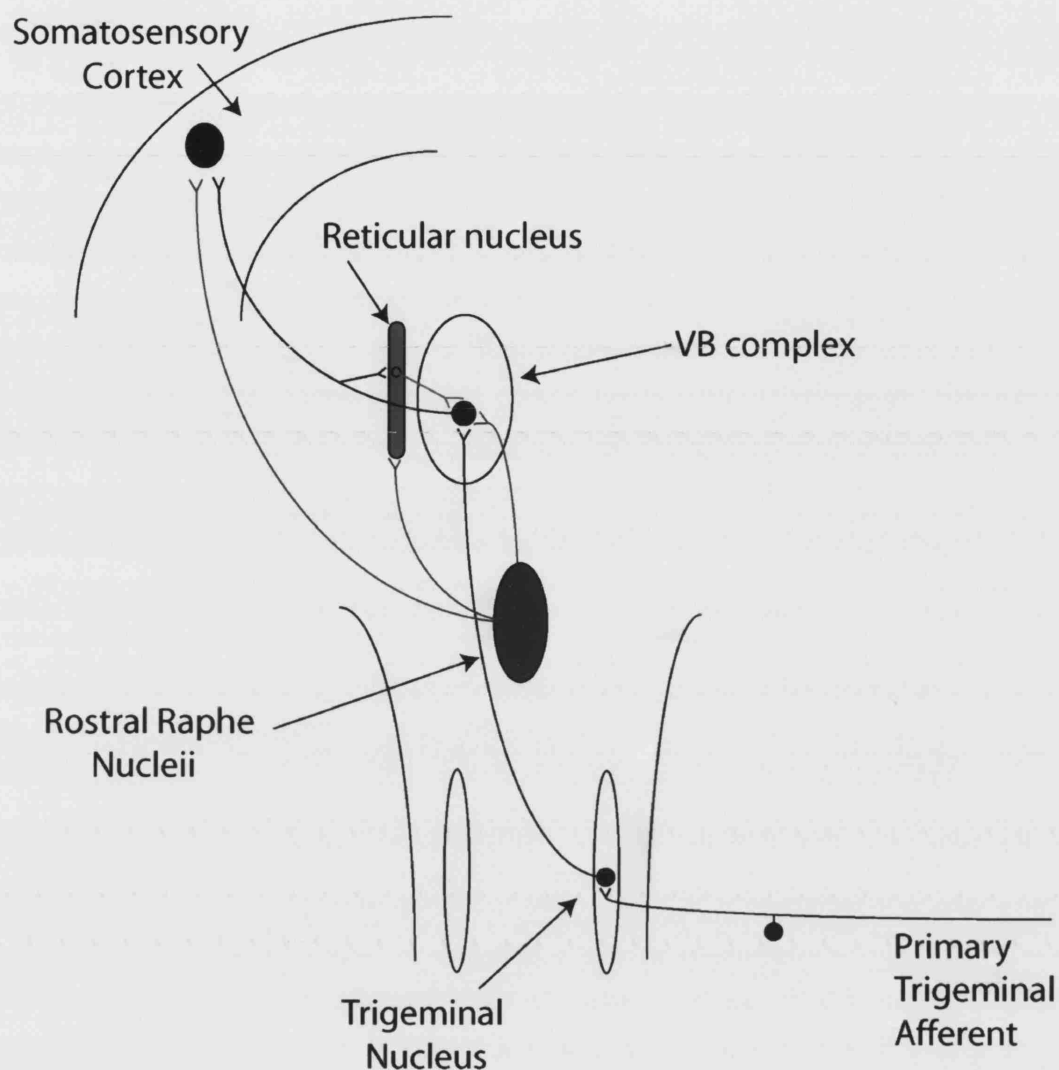


Figure 8: Ascending projections from the rostral raphe nuclei

Projections from the rostral raphe nuclei modulate thalamocortical neurotransmission. The rostral raphe nuclei (such as the dorsal raphe nucleus) send projections to the VPM nucleus, the thalamic reticular nucleus and cerebral cortex. The effects of serotonin in the VPM nucleus appear to be complex, both facilitating and inhibiting sensory neurotransmission. This probably reflects differential effects of 5-HT₁ and 5-HT₂ receptor activation. Serotonin depolarises neurons in the reticular nucleus (GABAergic) through a 5-HT₂ receptor action. Second order neurons in the trigeminal nucleus receive descending 5-HT innervation from the caudal raphe nuclei such as the nucleus raphe magnus (NRM) (not shown).

Serotonergic modulation of sensory neurotransmission in the thalamus

Serotonin appears to exert complex actions on thalamic neurons *in vivo*. Microiontophoretically applied serotonin inhibits the spontaneous firing of rat parafascicular neurons in a dose dependent manner. The response following noxious stimulation was also reduced (Andersen and Dafny, 1982). Results following microiontophoresis in the VB complex however indicate a dual action of serotonin. 5-HT has both facilitatory and inhibitory actions (Andersen and Curtis, 1964; Eaton and Salt, 1989; Phillis and Tebecis, 1967). This is consistent with the dual effects of serotonin throughout the CNS, often even involving the same neuron (Lee et al., 1985), and probably reflecting activation of different 5-HT receptors (Aghajanian and Andrade, 1997). 5-HT₁ receptor activation effects this response in the thalamus through regulation of hyperpolarising K⁺ channels (Aghajanian and Andrade, 1997; McCormick and Pape, 1990; Monckton and McCormick, 2002), though the magnitude of this response appears to vary between thalamic nuclei, and may vary across species (Monckton and McCormick, 2002).

Evidence for an ascending serotonergic modulatory system

Electrophysiological and biochemical studies support the existence of a supraspinal ascending serotonergic modulatory system (Fig. 6). Depletion of brain 5-HT following sectioning of the medial forebrain bundle (MFB) results in a reduction of pain thresholds (Harvey and Lints, 1971). Microinjection of opiates into the PAG results in elevated levels of 5-HT metabolites in the telencephalon (Algeri et al., 1980), while injection into the dorsal raphe depresses the responses of ventrobasal neurons to noxious stimulation (Kayser et al., 1983). More recently it has been demonstrated that the basal release of 5-HT is reduced in the VB complex of rats in a model of neuropathic pain (Goettl et al., 2002). The modulatory action of serotonin is not confined to the somatosensory system. Activity within the sensorimotor cortex also appears to be modulated by serotonergic activity within the thalamus (Storozhuk et al., 1995). Electrical stimulation of the DRN can modulate both spontaneous and nociceptive induced activity in thalamic parafascicular neurons. This still occurs following spinal transection (Qiao and Dafny, 1988), though selective depletion of serotonin by local administration of 5,7-dihydroxytryptan (5,7-DHT) – a serotonergic

neuronal toxin - attenuated this action (Andersen and Dafny, 1983). There is evidence that other thalamic sensory relay nuclei may receive 5-HT modulatory inputs from the dorsal raphe nucleus (Marks et al., 1987; Yoshida et al., 1984). Electrical stimulation of the dorsal and median raphe nuclei elicits metabolic changes in several thalamic and cortical regions in rats, some of which are involved specifically in the processing of pain from the head and face (Cudennec et al., 1987; Cudennec et al., 1988). Stimulation of the PAG in rats also has a dual modulatory action on VB neurons, both facilitating and inhibiting nociceptive transmission (Emmers, 1979).

Ergots and triptans

Ergots

Ergots have been used for the acute treatment of migraine for nearly 100 years (Koehler and Isler, 2002). Two are used in clinical practice – ergotamine tartrate and dihydroergotamine (DHE) (Silberstein, 1997). Ergometrine is an ergopeptide consisting of D-lysergic acid linked to a tricyclic peptide moiety. DHE is produced by selectively saturating the C₉=C₁₀ double bond in the parent D-lysergic acid moiety. This confers different binding and clinical properties. Both compounds bind to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT₂, α_1 and α_2 adrenoceptors and D₂ dopamine receptors (Silberstein, 1997) (Table 6). DHE is a more potent α -adrenergic blocker than ergotamine. Ergots inhibit trigeminovascular nociception in the trigeminocervical complex following intravenous and microiontophoretic administration (Storer and Goadsby, 1997). Ergotamine has poor and erratic oral bioavailability with slow absorption following oral dosing. It is often administered rectally in clinical practice to speed absorption and avoid first-pass hepatic metabolism. It frequently produces nausea and may cause daily headache if taken frequently (Davidoff, 2002). DHE may be given parenterally – the most common route is intravenously - and also intranasally. Absorption and bio-availability are predictable and it has a half-life ($t_{1/2}$) of about 10h – much longer than the triptans (Davidoff, 2002; Saxena and Tfelt-Hansen, 2000a). This may explain the low rates of headache recurrence observed following DHE treatment (Saadah, 1992a; Saadah, 1992b; Winner et al., 1993; Winner et al., 1996). Parenteral DHE may also be useful in the management of medication overuse headache and status migrainosus (Raskin, 1986; Raskin, 1990).

Triptans

The following discussion has been adapted from Sanchez del Rio and Moskowitz, (2000) and Saxena and Tfelt-Hansen (2000b). Sumatriptan was the first triptan to enter clinical practice and since then six additional “second” generation triptans have become commercially available. These are brain penetrant, with more favourable side effect profiles and pharmacokinetics. The triptans are very effective migraine abortive agents (Ferrari et al., 2002; Halpern et al., 2002; Hu et al., 2002; Nappi et al., 1994; Pascual and Cabarrocas, 2002). They can treat not only the headache but also relieve the nausea, vomiting, photophobia and phonophobia associated with an attack. They are effective against migraine with and without aura. If taken during the aura they are ineffective against the headache (Bates et al., 1994; Olesen et al. 2004). Sumatriptan may be given orally, rectally, intra-nasally and by sub-cutaneous injection. Naratriptan is a more lipophilic triptan with a higher oral bioavailability than sumatriptan – 95% vs. 58% in the dog (Connor et al., 1997). It has a longer plasma half-life in man ($t_{1/2}$ = 6h) and has a greater affinity for both 5-HT_{1B} and 5-HT_{1D} receptors. Naratriptan has between two to three times the potency of sumatriptan in models of canine artery vasoconstriction (Connor et al., 1997).

Table 6: Receptor binding affinities* of ergot alkaloids at human recombinant 5-HT₁ receptors

Receptor	Ergotamine	Dihydroergotamine
5-HT _{1A}	7.9	9.3
5-HT _{1B}	7.9	9.2
5-HT _{1D}	8.4	8.6
5-HT _{1E}	6.2	6.2
5-HT _{1F}	6.8	7.0
5-HT _{2A}	7.7	8.5

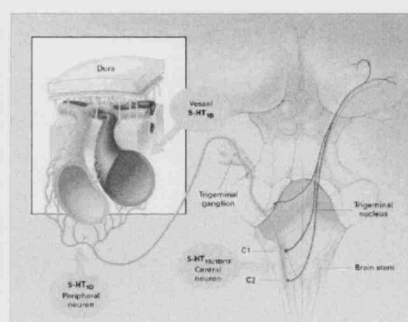
*Affinity expressed as either $-\log K_i$ or $-\log [IC_{50}]$ (nM); $-\log K_i$, negative logarithm of the concentration of drug required to inhibit binding to a receptor; $-\log [IC_{50}]$, negative logarithm of the concentration of antagonist required for 50% inhibition of agonist binding. Adapted from (Davidoff, 2002; Saxena and Tfelt-Hansen, 2000a)

Table 7: Receptor binding affinities* of triptans at human recombinant receptors

Receptor	Sumatriptan	Naratriptan	Zolmitriptan	Rizatriptan	Eletriptan	Frovatriptan	Almotriptan
5-HT _{1A}	6.0-6.9	7.1	6.5	6.3	7.4	7.3	7.4
5-HT _{1B}	7.4-8.1	8.5-8.7	8.3	7.4-8.0	8.0	8.0-8.6	7.2-8.0
5-HT _{1D}	7.9-8.5	8.3-8.5	9.2	8.0-8.4	8.9	8.4-8.9	8.0
5-HT _{1E}	5.8-7.6	7.2	<5.0	6.5-6.8	7.3	<6.0	-
5-HT _{1F}	7.6-7.9	8.3	7.1-7.6	6.6	8.2	7.0-8.0	-

*Affinity expressed as either $-\log K_i$ or $-\log [IC_{50}]$ (nM); $-\log K_i$: negative logarithm of the concentration of drug required to inhibit binding to a receptor; $-\log [IC_{50}]$: negative logarithm of the concentration of antagonist required for 50% inhibition of agonist binding. Adapted from (Davidoff, 2002)

The triptans are high affinity partial 5-HT_{1B/1D} agonists. Most of the members of the family also display high affinity binding for 5-HT_{1F} receptors, with less avid binding to 5-HT_{1A} and 5-HT_{1E} receptors (Table 7). The triptans have essentially no biological activity at other receptors. The triptans have modulatory actions at several points along the trigeminovascular nociceptive pathway (Tepper et al., 2002) (Fig. 7). Triptans may act as vasoconstrictors, activating 5-HT_{1B} receptors on the smooth muscle of cranial arteries. Alternatively activation of 5-HT_{1D} receptors located on trigeminal ganglion cells (Bonaventure et al., 1998) may inhibit the release of CGRP from peripheral trigeminal nerve endings. This peptide is a potent vasodilator and is pro-nociceptive. Triptans however are not able to block the effects of exogenous CGRP. Second generation triptans also act centrally to modulate firing of second order neurons in response to trigeminovascular nociceptive stimulation (Storer and Goadsby, 1997) – they do not require blood-brain barrier disruption unlike sumatriptan (Kaube et al., 1993a) to have effects in experimental animals.

**Figure 9: Triptans: possible loci of action**

Possible sites of action of triptans on intracranial blood vessels, first and second order trigeminovascular neurons. Taken from (Goadsby et al., 2002b).

The locus coeruleus and noradrenergic modulation

Adrenergic Receptors

Adrenergic receptors are divided into two distinct classes; called α and β receptors. All belong to the family of seven transmembrane G-protein coupled receptors. Three β -receptors are currently recognised (β_1 - β_3) and a possible fourth (β_4) receptor may exist. All are coupled to a G_s protein - activating adenylate cyclase (AC) with a resultant increase in intracellular levels of cAMP. Both β_1 and β_2 receptors are found throughout the CNS. So far β_3 receptor mRNA has only been demonstrated in the CNS, but it is widely distributed in the brain, especially in the hippocampus (Gibbs and Summers, 2000; Summers et al., 1995). α -Receptors are differentiated into two groups: α_1 and α_2 receptors. α_1 -Adrenoceptor activation modulates calcium metabolism through G_q regulation of phospholipase C (PLC). Four α_1 sub-types are recognised α_{1A} - α_{1D} . α_2 -Receptors are negatively coupled to AC through an inhibitory G_i protein - three sub-types have been described α_{2A} - α_{2C} (Cooper et al., 2003).

Adrenergic receptors are not static entities; they may change in both number and affinity following alterations in synaptic activity. β -Receptor density and sensitivity may be significantly altered following exposure to β -mimetic agents. Prolonged exposure to β -agonists leads to desensitisation, due to un-coupling of receptors from AC, which can be homologous or heterologous. Downregulation of receptors, where receptors are internalised/sequestered or alternatively gene transcription is reduced, also occurs. β -Antagonists will have the opposite effects, but the functional implications are poorly understood (Emilien and Maloteaux, 1998).

The locus coeruleus

Noradrenergic (NA) neurons are organised into groups of cells forming dorsal and ventral columns in the brainstem. These cell groups (each fulfilling specific functions) send projections throughout the CNS. In the pons the ventral column of NA cells lies in the ventrolateral reticular formation. It consists of the A5 and A7 cell groups. These send projections to the spinal cord and are responsible for modulating pain sensation and autonomic reflexes. The A6 cell group – also called the locus coeruleus (LC) - is found dorsolaterally on either side in the periventricular grey (Kandel et al., 2000) (Fig. 8). Lying below the floor of the ventrolateral aspect of the IV ventricle, a high concentration of melanin imparts a blue colour to the LC. Despite the fact that the LC

is the largest collection of NA neurons, each contains only about 1,500 cells in rats and 12,000 in humans (Cooper et al., 2003).

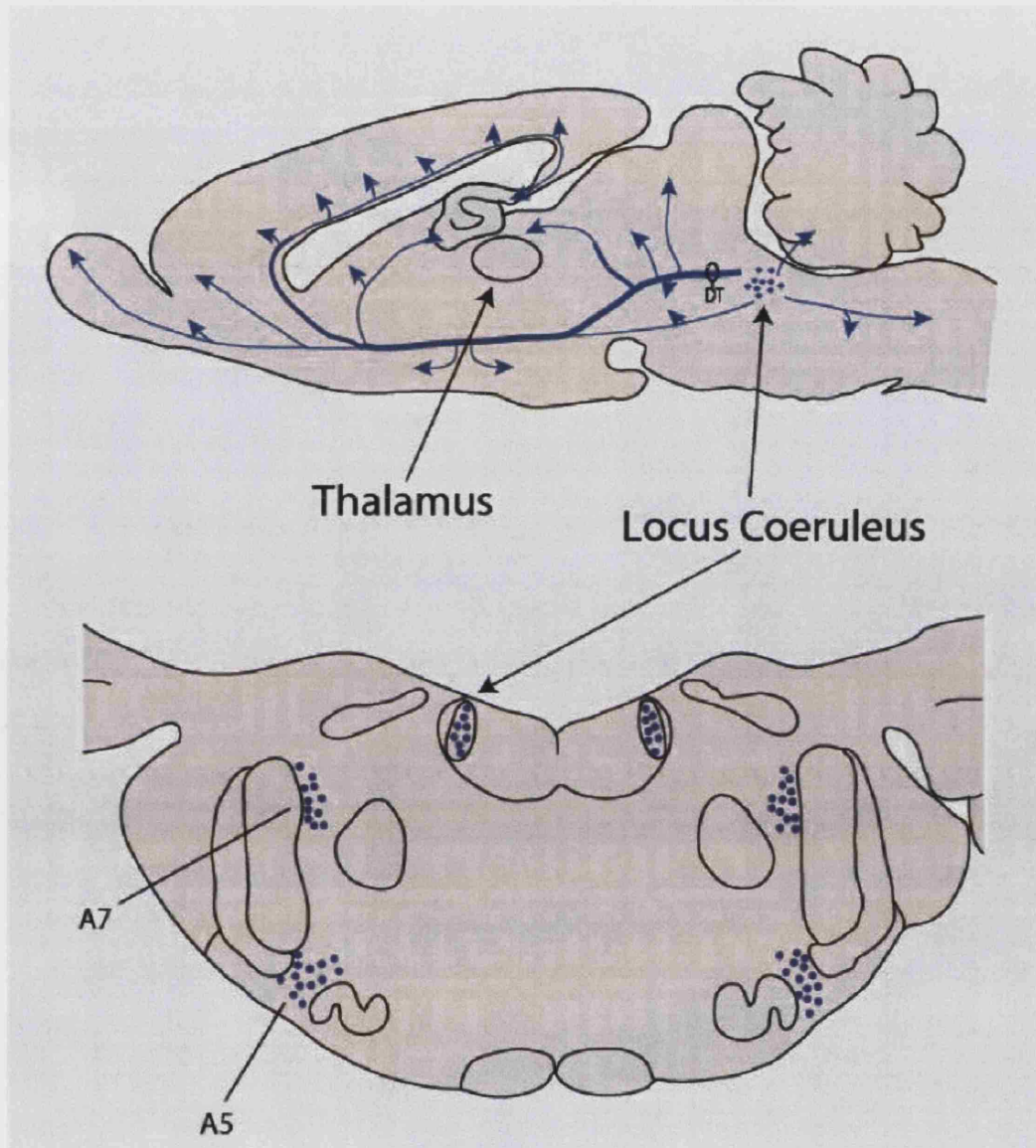


Figure 10: The locus coeruleus and its projections

Diagram of the locus coeruleus and its projections in the rat CNS. The loci coerulei (A6) are located in the dorso-lateral pontine tegmentum (lower panel). They send projections diffusely throughout the CNS, both rostrally and caudally (upper panel). Noradrenergic projections to the thalamus branch off from the dorsal tract (DT) before entering the thalamus in association with medial lemniscal afferents. Adapted from (Kandel et al., 2000).

These cells are neurochemically complex with evidence of acetylcholine (ACh), galanine (GAL), neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) within their cell bodies (Sutin and Jacobowitz, 1991). The LC sends extensive projections to the cerebral cortex, cerebellum, brainstem and spinal cord (Grzanna and Fritschy, 1991). LC neurons exhibit two patterns of neuronal firing. Cells may discharge tonically, the rate varying according to the behavioural state of the animal, eg during REM sleep LC neurons are virtually quiescent. They also exhibit phasic bursting activity in response to sensory stimulation. Physiological changes, such as hypovolaemia or hypoglycaemia which normally activate the sympathetic nervous system do not significantly affect LC firing (Jacobs et al., 1991). LC neurons are consistently and vigorously activated by aversive or overtly threatening stimuli, but they are also activated by novel innocuous stimuli. This suggests that LC firing is linked to the “conspicuousness” of sensory stimulation – including its novelty, physical characteristics (intensity, contrast, speed of onset, aversiveness) and behavioural setting (expectation) (Grant et al., 1988; Sara and Segal, 1991).

Noradrenergic Innervation of the ventrobasal complex

Histologically the noradrenergic innervation of the ventrobasal thalamus appears more dense than that of the serotonergic system (Westlund et al., 1990). The NA content of the VB complex exceeds that of 5-HT by a ratio of approximately 4:1 (Kobayashi et al., 1974; Weil-Fugazza et al., 1986; Weil-Fugazza et al., 1988). Many studies have shown that the VB complex receives its NA input from the LC. Fibres course rostrally within the dorsal tegmental bundle before branching off to join the medial lemniscus and entering the lateral thalamus (Lindvall et al., 1974; Peschanski and Besson, 1984a; Swanson and Hartman, 1975). In the VB complex NA neurons form fine, loose networks with spherical varicosities making axo-axonic or axodendritic contacts (Ishikawa and Tanaka, 1977; Lindvall et al., 1974; Westlund et al., 1991).

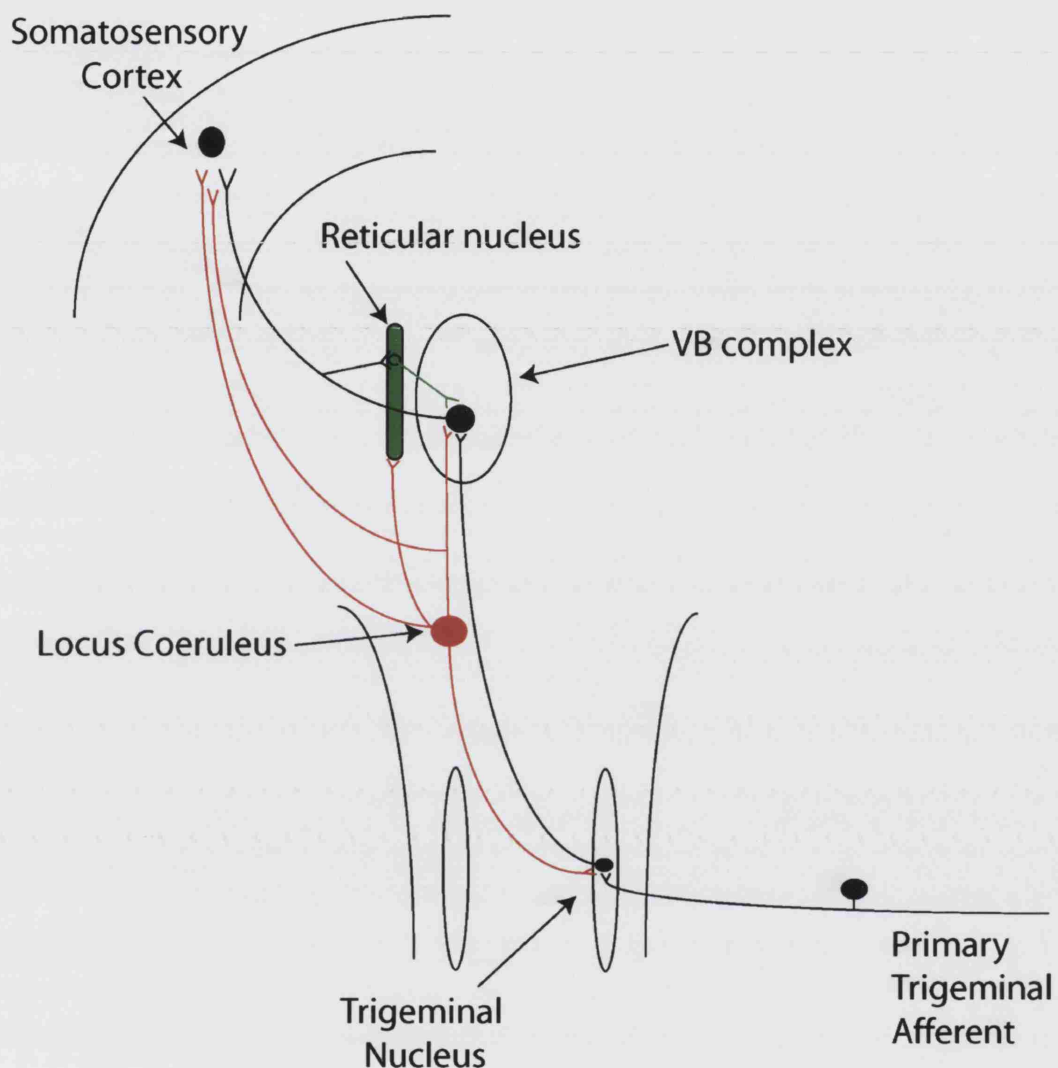


Figure 11: Coerulear projections to the trigeminal sensory pathway

Noradrenergic innervation of the trigeminal sensory pathway: the locus coeruleus may influence the transmission of trigeminovascular nociceptive neurotransmission at several points including the medullary dorsal horn, thalamus and probably also the cortex. In the diencephalon the VPM and the reticular nuclei receive a dense noradrenergic innervation. Like serotonin, noradrenaline appears to have complex effects on sensory neurotransmission in the VPM (both facilitating and inhibiting), while having largely facilitatory effects on reticular neuronal firing (α_2 adrenoceptor action).

Coerulear projections appear to be highly organised, targeting multiple sites along the somatosensory pathway from the contralateral side of the face. Thalamic efferents originate largely from the caudal pole of the LC. The projection to the VB complex is predominantly ipsilateral. A significant proportion of the LC cells sending projections to the trigeminal somatosensory thalamus also send axon collaterals to the ipsilateral somatosensory cortex (Simpson et al., 1997) (Fig. 9). When we consider that the coerulear efferents to the principal trigeminal nucleus (Vp) arise from the contralateral LC it appears that NA may be able to modulate the flow of trigeminal sensory information simultaneously at several points between Vp and SI - II (Simpson et al., 1997).

β -Adrenoceptors in the Thalamus

In the rat β -adrenoceptors are found in most thalamic nuclei (Bylund and Snyder, 1976). Quite high levels have been recorded in the VB complex. These are predominately β_1 receptors (Booze et al., 1989; Rainbow et al., 1984) – the ratio of β_1 : β_2 receptors has been estimated to be roughly 4:1 (Rainbow et al., 1984). The distribution and make-up of the β -receptor population in the thalamus is species related. The guinea pig VPM has a lower total β -receptor density than the rat, but the relative proportions of β_1 : β_2 receptors are approximately equal (Booze et al., 1989). β_3 receptors have yet to be demonstrated (Summers et al., 1995). In humans β -receptors are found homogeneously throughout the thalamus both histologically in high concentrations (Pazos et al., 1985; Reznikoff et al., 1986) and using functional imaging techniques - though only low levels have been visualised (van Waarde et al., 1997).

β -Blockers and migraine prophylaxis

The following discussion has been adapted from Davidoff (2002), Emilien and Maloteaux (1998), Hoffman and Lefkowitz (1990) and Tfelt-Hansen and Shanks (2000). Since the serendipitous discovery of their efficacy in migraine during the 1960s (Rabkin et al., 1966), β -adrenoceptor antagonists have become the agents of first choice for migraine prophylaxis. Five β -blockers: propranolol, atenolol, metoprolol, timolol and nadolol have proven efficacy for reducing the frequency of migraine attacks (Andersson and Vinge, 1990; Tfelt-Hansen and Shanks, 2000).

β -Blockers are stereoselective antagonists of β -adrenoceptors. They may be further characterised by receptor selectivity and intrinsic sympathomimetic activity (ISA) (Emilien and Maloteaux, 1998). Selectivity refers to the relative ability of a β -blocker to antagonise the actions of NA at β_1 and β_2 receptors – such selectivity however is often dose dependent, being lost at higher concentrations. Nonselective agents (both β_1 and β_2) include: propranolol, timolol and nadolol, while atenolol and metoprolol are relatively β_1 selective. β_2 antagonism accounts for many of the undesirable side-effects of β -blockers eg bronchospasm and impotence. Certain β -blockers, such as pindolol, are partial agonists. In the setting of low noradrenergic activity they behave as weak agonists with ISA. These β -blockers however do not appear to be effective migraine preventatives (Andersson and Vinge, 1990). In addition to β -receptor antagonism some β -blockers have local anaesthetic-like membrane stabilising-properties. Of the drugs used for migraine only propranolol exhibits this property, though it is unlikely that this contributes to its therapeutic actions at physiological doses. Aryloxyalkylamine β -blockers are also antagonists at human serotonergic 5-HT_{1A} receptors (Nishio et al., 1989), but this does not appear to correlate with prophylactic efficacy (Newman-Tancredi et al., 1997) (Table 8).

Propranolol is highly lipophilic; it is readily absorbed following oral administration but is subject to extensive first-pass metabolism. Oral bioavailability is approximately 25%, but there is a great deal of inter-individual variability - plasma concentrations vary twenty-fold after oral administration. Propranolol is rapidly metabolised, resulting in a short plasma half-life ($t_{1/2} = 4$ h). Conventional propranolol must therefore be administered two to four times a day to achieve stable plasma levels. The slow-release formulation has a much longer half-life ($t_{1/2} = 10\text{-}20\text{h}$) and produces sustained plasma levels with less variations. It has a lower bioavailability and so higher total daily doses are needed. Atenolol by contrast is hydrophilic but its absorption is incomplete and it penetrates the CNS less readily. It is not as extensively metabolised as propranolol. Oral bioavailability is therefore higher (50%) with less variability in plasma concentrations following oral dosing. The plasma half-life of atenolol is between 5 – 8 hours and once daily dosing is often sufficient.

Table 8: Properties of β -adrenoceptor blockers

β-Blocker	Efficacy in migraine	CNS penetration	Membrane stabilising activity	β_1 selectivity	Partial agonist activity	Affinity for 5-HT receptors	t_{1/2} (hours)	Bioavailability (%)
Propranolol	yes	yes	yes	no	no	high	3-6	30
Pindolol	no	yes	yes	no	yes	high	3	90
Timolol	yes	yes	no	no	no	NA	4	50
Acebutalol	no	yes	yes	yes	yes	NA	3-4	40
Metoprolol	yes	yes	no	yes	no	NA	3-4	35
Atenolol	yes	poor	no	yes	no	low	5-8	40

Adapted from (Tfelt-Hansen and Shanks, 2000)

Voltage-gated calcium channels

Calcium ions (Ca^{2+}) play a fundamental role in neuronal function. They modulate membrane excitability and underlie neural phenomena such as central sensitisation (Cooper et al., 2003). They are critical for initiating neurotransmitter release, influencing second messenger concentrations and also regulating gene expression. Intra-cytoplasmic calcium levels are controlled by regulating ion flux through the following channels:

- a) voltage-gated calcium channels
- b) ligand-gated non-specific cation channels
- c) receptor-activated calcium channels (Prado, 2001)

Calcium may be mobilised from intracellular stores or enter the neuron from the extracellular space. Voltage-gated/dependent calcium channels (VDCC) are activated by neuronal depolarisation. One classification system (Tsien et al. 1988), which will be used in this thesis, groups them into six classes: L, N, P, Q, R and T according to their electrophysiological properties and pharmacological susceptibility to specific blocking agents (Table 9). At the most basic level voltage gated calcium channels may be divided electrophysiologically into two groups depending on their threshold of activation. Low-threshold VDCCs (T-type VDCCs) are activated at more hyperpolarised membrane voltages relative to high-threshold (L-, N-, P-, and Q-type) channels. These require a greater degree of membrane depolarisation before they are activated (See references in Catterall, 2000).

VDCCs are composed of α_1 , $\alpha_2\delta$, β and γ subunits. The α_1 subunit contains the selective calcium ion pore, a voltage sensor and the binding site for selective high-threshold VDCC blockers. Ten isoforms of the α_1 sub-unit have been identified and this forms the basis for the most recent classification system (Ertel et al., 2000). The ten isoforms are grouped into three families on the basis of their sequence homology (Table 9). Within each family of α_1 sub-units there is a high degree of amino acid sequence communality. There is a marked dichotomy however between groups (for example groups 1 and 2 share 40% sequence homology but group 3 (low-threshold VDCCs) shares only 25% with the two other groups). The other sub-units regulate to varying degrees the level of α_1 sub-unit expression, voltage dependence and activation kinetics (Catterall, 2000).

High threshold voltage dependent calcium channels are differentially distributed at the neuronal level. P/Q-type VDCCs are located predominantly at presynaptic sites (Westenbroek et al., 1998; Westenbroek et al., 1995), N- are found both pre- and post-synaptically (Westenbroek et al., 1992) while L-type channels are mainly located on the proximal dendrites and soma of neurons (Hanson and Smith, 2002; Westenbroek et al., 1990).

Synaptic release of neurotransmitters is dependant on influx of calcium ions through voltage gated calcium channels. P/Q-type VDCCs appear to be the most prevalent exocytotic calcium channel within the CNS (Dunlap et al., 1995) controlling the release of excitatory amino acids, monoamines and peptide neurotransmitters (Luebke et al., 1993; Pocock and Nicholls, 1992; Takahashi and Momiyama, 1993; Turner et al., 1993). Exocytotic release of a variety of neurotransmitters is also inhibited by the N-type blocker ω -conotoxin G VIa (Dickie and Davies, 1992; Luebke et al., 1993; Santicioli et al., 1992; Takahashi and Momiyama, 1993; Turner et al., 1993). Blockade of N-type channels appears to be more effective at blocking inhibitory rather than excitatory synaptic transmission (Dunlap et al., 1995). Excitatory glutaminergic neurotransmission is not completely blocked by application of ω -conotoxin G VIa, indicating that several VDCCs are involved in neurotransmitter release (Takahashi and Momiyama, 1993; Turner et al., 1993). Blockade of one type of calcium channel may therefore not significantly affect neurotransmission especially if the presynaptic neuron is strongly depolarised. L-type channels do not appear to have a very significant role in neurotransmitter release in the CNS, though the nature of the stimulus used to evoke neurotransmitter release may be crucially important;

nifedipine may inhibit release of Substance P from dorsal root ganglion cells when they are depolarised with KCl but not electrical stimulation (Holz et al., 1988). Being located on the post-synaptic neuron L-type VDCCs, along with N-type channels, may have a more important role regulating neuronal membrane properties and synaptic integration.

High-threshold VDCCs and nociception in the spinal cord

Blockade of N-type, P/Q-type and to a lesser extent L-type channels appears to modulate spinal nociception. The effects though may vary depending on the nature of the nociceptive stimulus and whether peripheral and central sensitisation have become established (Vanegas and Schaible, 2000). The following discussion is adapted in part from Vanegas and Schaible (2000).

Table 9: VDCC α_1 subunit properties

Threshold of activation	Ca ₂₊ channel	Ca ₂₊ current type	Previous name of α_1 subunits	Specific blockers	Primary localisations
High threshold	Ca _v 1.1	L	α_{1S}	Dihydropyridines	Skeletal muscle
	Ca _v 1.2	L	α_{1C}	Dihydropyridines	Cardiac muscle Endocrine cells Neurons
	Ca _v 1.3	L	α_{1D}	Dihydropyridines	Endocrine cells Neurons
	Ca _v 1.4	L	α_{1F}	None	Retina
	Ca _v 2.1	P/Q	α_{1A}	ω -Agatoxin IVA	Nerve terminals Dendrites
	Ca _v 2.2	N	α_{1B}	ω -Conotoxin GVIA	Nerve terminals Dendrites
	Ca _v 2.3	R	α_{1E}	SNX-482?	Cell bodies Dendrites Nerve terminals
Low threshold	Ca _v 3.1	T	α_{1G}	Ethosuximide	Cardiac muscle Skeletal muscle Neurons
	Ca _v 3.2	T	α_{1H}	Ethosuximide	Cardiac muscle Neurons
	Ca _v 3.3	T	α_{1I}	Ethosuximide	Neurons

Adapted from (Catterall, 2000)

L-type VDCCs

Antagonists of L-type VDCCs have had equivocal effects following spinal administration in models of acute, short-lasting somatic nociception (Del Pozo et al., 1987; Hara et al., 1998; Malmberg and Yaksh, 1994; Neugebauer et al., 1996; Sluka, 1997; Sluka, 1998). Unfortunately as many studies show a conspicuous lack of effect,

as those demonstrating an inhibitory action. L-type VDCCs may have a more important role in visceral nociceptive neurotransmission (Del Pozo et al., 1987; Hara et al., 1998; Miranda et al., 1992) though these results have not being replicated by all studies (Horvath et al., 2001).

L-type channels may have a role in chronic spinal nociception (using models of chemical irritation of peripheral nerves or articular inflammation). Two behavioural phases are noted in these models, early and late. The late phase correlates with the onset of central sensitisation (Dickenson and Sullivan, 1987). Though L-type VDCCs have a doubtful role in the early phase, they do have a modest role in the late inflammatory phase (Coderre and Melzack, 1992; Malmberg and Yaksh, 1994; Neugebauer et al., 1996).

N-type VDCCs

Antagonists of N-type channels consistently prevent reactions to acute nociceptive stimuli, such as mechanical or thermal stimulation (Malmberg and Yaksh, 1994; Nebe et al., 1998; Neugebauer et al., 1996), though negative effects have also been noted (Sluka, 1997). Both early and late responses to chronic nerve irritation are reduced by pre-treatment with N-type blockers. Once central sensitisation has become established they are still effective at reducing nociceptive behaviour - both primary and secondary hyperalgesia and allodynia (Diaz and Dickenson, 1997; Nebe et al., 1998; Neugebauer et al., 1996; Sluka, 1997; Sluka, 1998). N-type VDCC therefore appear to have an unequivocal role in mediating nociceptive neurotransmission in the spinal cord.

P/Q-type VDCCs

As P/Q-type channels are involved in both excitatory and inhibitory synaptic neurotransmission (Dunlap et al., 1995; Luebke et al., 1993; Takahashi and Momiyama, 1993; Turner et al., 1993), it is therefore not a surprise that P/Q-type channel blockers are reported to have inhibitory, facilitatory or even no effects on spinal neurons following brief nociceptive stimulation (Nebe et al., 1997; Sluka, 1997; Sluka, 1998). Primary and secondary hyperalgesia resulting from chronic inflammation however is prevented by pre-treatment with P/Q- blocking agents (Diaz and Dickenson, 1997; Malmberg and Yaksh, 1994; Nebe et al., 1997; Sluka, 1997). This suggests that P/Q-type channels have an important role in the development of

central sensitisation. Once central sensitisation becomes established their role in transmitting nociceptive information is more restricted (Nebe et al., 1997; Sluka, 1998).

Though it cannot be assumed that the same array of high threshold VDCCs are involved in spinal somatic and trigeminovascular nociception, initial studies do indicate that N-, P/Q- and L-type channels have a role in transmitting sensory information from the dura (Ebersberger et al., 2004). Blockade of N-type channels effectively inhibit the responses of neurons in the spinal trigeminal nucleus to cold and inflammatory stimulation of the dura. Blockade of P/Q-type channels have a less profound effect, while L-type block does not have a significant action. Interestingly in both cases application of L- and P/Q-type VDCC blockers produce an increase in spontaneous firing rates, suggesting a disinhibitory action on dorsal horn interneurons. As VDCC blockers were applied to the exposed brainstem and upper cervical cord, the effect of these compounds was quite generalised. It is not possible to exclude the possibility that these effects were the result of actions at other sites such as the PAG (Knight et al., 2002).

The lack of effect of L-type channel blockers in the trigeminal nucleus is in contrast with their action on neurogenically induced dural vasodilation (Akerman et al., 2003). The potent L-type channel blocker calciseptine inhibits dilation of meningeal arteries. It is suggested that this was due to the inhibition of CGRP release from trigeminovascular neurons. P/Q-type and N-type VDCC blockers have a similar action. This may suggest that these channels are important in the regulation of trigeminovascular neurons in the periphery. Alternatively this may represent a direct action on vascular smooth muscle which is unrelated to an action on nociceptive neurons.

Calcium Channel blockers and migraine prophylaxis

The VDCC blockers/modulators are a heterogeneous group of drugs; all have the common effect of blocking the influx of calcium ions through high threshold voltage gated calcium channels. Five classes of calcium-blockers have been used for migraine prophylaxis with varying levels of success:

a) phenylalkylamines e.g. verapamil

- b) dihydropyridines e.g. nifedipine and nimodipine
- c) benzothiazepines e.g. diltiazem
- d) diphenylalkylamines e.g. flunarazine
- e) organic acid derivatives e.g. gabapentin (this is strictly a VDCC modulator)

With the exception of gabapentin and flunarazine, convincing evidence in support of the efficacy of calcium-blockers in migraine is lacking (Andersson and Vinge, 1990; Toda and Tfelt-Hansen, 2000). Most studies have been performed on only small numbers of patients and suffered from high drop-out rates. The beneficial effects of calcium channel blockers may be delayed for some time (perhaps up to several months in the case of flunarazine) which has important implications for compliance. Their mechanism of action is unknown but it is unlikely that their vasodilatory or cytoprotective actions have a significant role in migraine prophylaxis. It is suggested that a reported inhibition of cortical spreading depression indicates a specific effect against migraine aura.

Gabapentin has been studied in two randomised placebo-controlled trials to date. In both cases it produced a reduction in headache (Di Trapani et al., 2000; Mathew et al., 2001). Gabapentin is a derivative of GABA with an additional cyclohexyl group (but it has no actions against GABA receptors). It modulates voltage-gated calcium influx in dorsal root ganglion neurons (Sutton et al., 2002). It is not known however if other $\alpha_2\delta$ modulators (such as pregabalin) will also be effective in the treatment of migraine. Flunarazine is an effective migraine prophylactic agent (Andersson and Vinge, 1990; Sorensen et al., 1986) for both adults and children. It has other pharmacological properties however in addition to its ability to block L-type VDCCs, including actions on the serotonergic, dopaminergic and histaminergic neurotransmitter systems.

Proposed experiments

Migraine is certainly not just a disorder of head pain. As already discussed migraineurs complain of other debilitating symptoms including photo- and phonophobia. Any attempt to explain the pathogenesis of migraine must try to provide a unifying basis for all these symptoms. The thalamus and cortex are the two sites where neuronal dysfunction could result in abnormal sensory perception involving

multiple modalities. At present however nociception remains the sensory modality most amenable to study *in vivo*. It is possible that trigeminal neurons, activated by nociceptive stimulation of intracranial vascular structures are also activated during migraine attacks. Knowledge of the pharmacological mechanisms involved in the transmission of nociceptive information within this pathway may therefore prove useful in future drug development. Afferent trigeminovascular sensory information is relayed in the trigeminocervical complex (TCC) and also the VPM nucleus of the thalamus enroute to the primary somatosensory cortex. In the following four studies aspects of the pharmacology of trigeminovascular nociceptive neurotransmission will be studied in the TCC of the cat and rat VPM using the technique of microiontophoresis. In each study two types of experiment were attempted. The first examined the ability of a compound to modulate the response of a post-synaptic neuron, be it second or third order, to trigeminovascular nociceptive stimulation. The chosen stimulus was electrical stimulation of the superior sagittal sinus. The next looked more specifically at modulation of action potential firing by neurons in response to local microiontophoretic delivery of L-glutamate. The studies were not designed to be exhaustive pharmacological examinations; rather they were conceived with a specific clinical question in mind.

Microiontophoresis has been extensively used to study modulation of trigeminal nociception in the cat TCC. No study to date has examined the role of voltage gated calcium channels in nociceptive neurotransmission specifically within the TCC. P/Q-type VDCCs have been implicated in the pathogenesis of Familial Hemiplegic Migraine and calcium channel blockers are used for the prophylactic treatment of migraine. Calcium channels therefore appear to have an important role in the pathogenesis of migraine but it is not clear whether all VDCCs are equally involved and where their principal site of involvement might be. High threshold VDCCs have been studied in great detail using various models of chronic neuropathic and inflammatory pain however it is not known whether these ion channels also modulate trigeminal nociception within the medullary dorsal horn. Study 4 addressed this question using peptide blockers of specific VDCCs. A previous study (Ebersberger et al., 2004) applied peptide blockers to the entire brainstem and upper cervical cord. This approach lacked anatomical localisation of drug effect – a difficulty which microiontophoresis is ideally suited to overcome.

Most of this thesis will concentrate on modulation of thalamic neurotransmission. Though we know that trigeminovascular nociceptive information is relayed in the ventrobasal complex of the thalamus; little is known about the pharmacology of its neurotransmission. The thalamus is a logical site for research as Migraine may be a centrally driven process involving abnormal monoaminergic regulation of sensory neurotransmission. Though the sensory relay nuclei of the thalamus receive a variety of modulatory inputs (including GABA, 5-HT, NA, ACh, Histamine), these studies were designed to take advantage of knowledge derived from clinical practice. Some of the most effective anti-migraine agents currently available may have the potential to influence the modulatory actions of several neurotransmitters in the thalamus. We explored this using sodium valproate, propranolol and naratriptan and attempts were made to define the pharmacological mechanisms of their actions.

Methods

“It ought to be generally known that the source of our pleasure, merriment, laughter, amusement, as of our grief, pain, anxiety and tears, is none other than the brain.”

Hippocrates (460-375 BC)

Animals

All experiments were conducted under terminal anaesthesia in accordance with a project license and guidelines issued by the Home Office of the United Kingdom of Great Britain & Northern Ireland under the Animals (Scientific Procedures) Act 1986. The animals used in these experiments were: 91 male Sprague Dawley rats (A. Tuck and Sons, U.K) weighing 316 ± 6 g (mean \pm SD) and 23 cats of mixed sex (16 male, 7 female) (Denny Brown Laboratories, Institute of Neurology, Queen Square, London, UK) weighing 3.6 ± 0.3 kg (mean \pm SD). All animals were maintained on a 12-hour light/dark cycle. Rats were given food and water *ad libitum*. Cats were given sufficient food to meet their nutritional needs but water was freely available. They were however kept fasting from midnight on the day of surgery

Rat experiments

Anaesthesia

In all experiments anaesthesia was induced with intraperitoneal pentobarbitone sodium (Sagatal[®], Rhone Merieux, Harlow, Essex) 60mg/kg and maintained with supplementary doses of intravenous pentobarbitone sodium (30 mg/kg). A sufficient depth of anaesthesia was judged from the absence of withdrawal reflexes, and gross fluctuations in blood pressure. The depth of anaesthesia was not monitored by electroencephalography (EEG) in these experiments. We did however use physical signs and receptive field properties to estimate the stages of anaesthesia as described by Guedel. Though physical signs are less discriminating than the EEG for determining the stage of anaesthesia, most of our recording likely took place within stages III-3 and III-4 (Friedberg et al., 1999). At the end of each experiment animals were given a lethal dose of pentobarbital sodium (Lethobarb[®], 200mg/ml – 1ml, Fort Dodge Animal Health, Southampton, UK).

Surgery

The left femoral artery and vein were exposed and separated using blunt dissection. They were cannulated with lengths of polythene tubing (external diameter 0.96 mm, Portex Ltd., Kent, UK) and secured with braided silk (Look[®], Surgical Specialities Corp., Reading, PA, USA). The patency of each cannula was confirmed by a free

backflow of blood and was maintained by periodic flushes of isotonic saline (Phoenix Pharmaceuticals, Gloucester, UK). The trachea was exposed by blunt dissection and intubated with an endotracheal tube made from a modified polythene venous catheter (Portex Ltd, Kent, UK). The distal trachea was tied off and the endotracheal tube was secured with braided silk sutures.

Following cannulation of the trachea and femoral vessels, rats were placed in a stereotaxic head frame (David Kopf Instruments, Tujunga, CA, USA – Model 1600). The skin of the dorsal surface of the skull was divided with a 2.5 cm midline incision and the bone exposed. The edges of the skin were held apart with sutures and the right masseter muscle was separated from its bony insertion on the parietal bone. A rectangular window (approximately 4 x 6 mm) was drilled in the superior surface of the right parietal bone using a dental drill (Volvere GX[®], Nakanishi Dental, Japan). This straddled the mid-line, and its rostral margin was approximately 2mm caudal to bregma. The bone was cooled by constant irrigation with isotonic saline during drilling to prevent heat damage to underlying structures. The rectangular section of bone was then gently lifted away from the intact dura, exposing the parietal cortex and the superior sagittal sinus. Any bleeding was staunches by gentle application of small cotton wool pellets (Richmond Dental, Charlotte, NC, USA). The site was covered with warm mineral oil (BDH Laboratory Supplies, Poole, UK) to prevent dehydration. The dura mater was carefully incised and reflected to expose the underlying parietal cortex just before the microiontophoretic recording electrode was lowered into the thalamus. This is demonstrated schematically in Fig. 10 and 14.

Animal Maintenance

Throughout the surgery and subsequent experiments the core temperature of the animals was monitored with a rectal thermometer and maintained between 36.5°C and 38°C by a homoeothermic blanket system (TC-1000, CWE Inc., Ardmore, USA). Blood pressure was continuously monitored via the femoral arterial line which was connected to a saline filled transducer (Ohmeda DTX Plus pressure transducer with PM-1000 transducer amplifier from CWE Inc.). During experiments animals were paralysed with the neuromuscular blocking agent pancuronium bromide (Pavulon[®], Organon, Cambridge, UK, 1 mg/kg initially, maintenance with 0.4 mg/kg as needed) and ventilated artificially (3-5mls, 60-80 strokes/minute (7025 Rodent Ventilator,

Ugo Basile, Varese, Italy)) with oxygen enriched room air (medical air (0.6L/min) and O₂ (0.6L/min) (BOC Medical, Manchester, UK)). End-tidal CO₂ was continuously monitored (Capstar-100 CO₂ Analyser, CWE Inc.) and maintained between 3.5% and 4.5% by altering either the rate of respiration or stroke volume. Physiological parameters and electrophysiological data were recorded (PCM-R500, Sony, Japan) onto digital tape (Maxell DDS 90s/2GB).

Placement of stimulating electrodes

Two platinum wire stimulating electrodes were placed onto the SSS using a micromanipulator (David Kopf, U.S.A.). Every effort was taken to minimise contact between the cortex and stimulating electrodes. Periodically they were repositioned to allow removal of any CSF that may have accumulated under the layer of electrically insulating liquid paraffin, fresh paraffin was then applied.

Microiontophoretic electrode filling

In all thalamic studies carbon-fibre seven-barrelled microiontophoretic electrodes were used (Carbostar 7S[®], Kation Scientific, Minneapolis, MN, U.S.A.). These consisted of a seven-barrelled glass pipette incorporating a carbon fibre recording electrode (Impedance @ 1 KHz: 0.4 – 0.8 MΩ), with an exposed tip length of approximately 10 μm. All solutions were filtered (Anotop 10[®], 0.02μm inorganic membrane filter, Whatman Int. Ltd., UK) to remove particulates. Barrels were filled at least one hour before use with non-metallic syringe needles (Microfil[®] 34G, WPI, Sarasota, USA). The proximal ends were covered (Parafilm M[®], American National Can, Chicago, USA) to reduce the risk of retrograde tracking of solutions and contamination of barrels prior to use. Early filling of the micropipette barrels before experiments tended to reduce barrel resistances.

Stereotaxis and identification of the ventroposteromedial nucleus

Electrodes were fitted to a hydraulic microdrive (Model 650 Micropositioner, David Kopf, USA) and then lowered into the region of the VPM according to the stereotaxic co-ordinates of Paxinos and Watson (Paxinos and Watson, 1986). L-Glutamate was ejected (20nA) while the electrode was lowered towards the VPM (to a depth of 5mm

using the micromanipulator) to reduce the risk of barrel blocking. At this depth the L-glutamate current was switched off and receptive fields on the head and face were sought as the electrode was slowly advanced using the micromanipulator. Mechanical stimulation of craniofacial receptive fields was used to gauge whether the electrode was in the VPM. Trigeminal afferents were then activated by stimulating the SSS with square-wave pulses to excite trigeminovascular afferents. Supra-maximal stimulating voltages were initially used but once a suitable unit was identified this was reduced to threshold levels (Grass Instruments S88 Stimulator, West Warwick, RI, U.S.A.; 6 – 30 V, 250 μ s, 0.5 Hz) to reduce the risk of current spread to the cortex. It was felt that this was more likely to occur at high stimulus voltages. The voltage necessary to activate sensory afferents varied between experiments, presumably due to differences in depth of anaesthesia and electrical contact. The microdrive was then used to advance the electrode in 5 μ m steps while we searched for trigeminovascular nociceptive responsive neurons within the VPM nucleus.

Equipment and settings used in electrophysiological recording

The signal from the recording electrode was fed via a head-stage amplifier (NL100AK, Neurolog, Digitimer, Herts, U.K) - the head-stage amplifier was grounded to the animal by a reference silver/silver chloride electrode inserted either into the dorsal neck muscles or subcutaneously – through an AC preamplifier (Neurolog NL104A, gain x1000) and noise eliminator (Humbug, Quest Scientific, North Vancouver, BC, Canada) to Neurolog filters (NL125, bandwidth approximately 700 – 10 kHz) and then to a second stage variable amplifier (Neurolog NL106, gain approximately x 50 – 90). This signal was also fed to a gated amplitude discriminator (Neurolog NL201). The filtered and amplified signal was displayed on an oscilloscope (OS 7020A, Goldstar Precision Co., Korea) and also fed to an audio amplifier (Neurolog NL120) to assist with the discrimination of single unit activity from background noise. A personal computer (Dell Computer Corporation, Berks, UK) running Spike® 2, version 4.15 software (Cambridge Electronic Design, Cambridge, UK), was used to collect and analyse data (Fig. 10). The stereotaxic frame - and table on which it was mounted - was grounded to the earth of the mains electricity supply. The cable from the head-stage amplifier to the AC preamplifier was covered in aluminium foil and grounded.

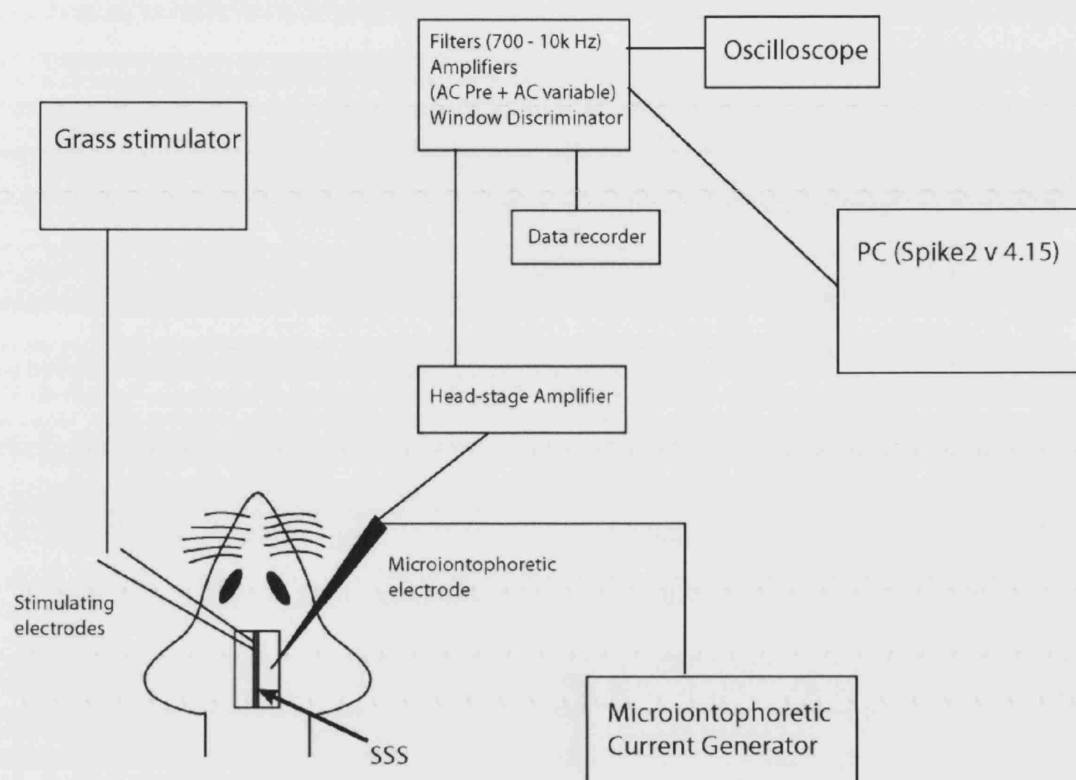


Figure 12: Circuit diagram of equipment used for electrophysiological recording

Schematic diagram of the cranial preparation and equipment used in experiments to record from neurons in the VPM. Stimulating electrodes are placed on the SSS following a craniotomy and a microiontophoretic combination electrode was placed in the VPM nucleus. The electrical recording made from a VPM neuron was fed through a series of amplifiers and filters to a personal computer for analysis.

Receptive fields

Receptive fields were sought on the contralateral craniofacial region for all cells. For those cells with a receptive field not involving the vibrissae, non-noxious stimuli were provided by gentle brushing with a blunt probe, while noxious stimuli were produced by pinching with toothed forceps. Cells were classified as low threshold mechanoreceptive (LTM) if they responded only to non-noxious stimuli, nociceptive specific (NS) if they responded to noxious stimulation only, and wide dynamic range

(WDR) if they responded to both. WDR cells generally had an increase in the rate of firing in response to noxious stimuli (Hu et al., 1981). The majority of cells however had receptive fields limited to the vibrissae and furry buccal pad. This is not surprising given the large volume dedicated to the representation of vibrissae within the rat VPM (Vahle-Hinz and Gottschaldt, 1983). With such cells each vibrissa was manipulated with a fine needle mounted on a probe, taking care not to deflect surrounding vibrissae, to find the whisker(s) that triggered a response upon deflection.

Principles and limitations of microiontophoresis

The following summary has been adapted from Stone (1985). Microiontophoresis utilises the flow of electric charge through an aqueous solution to eject drugs with a high degree of anatomical precision (Fig. 11). This technique will only work with compounds that are both water soluble and ionisable. In the case of compounds such as cadmium or valproic acid this is relatively easy as they are available as salts and readily dissociate in solution. With more complex organic molecules it is often necessary to add small quantities of acid or alkali to the solution. This exploits the presence of specific proton donor or acceptor moieties on the parent molecule - for example if a compound has an amine side-group (-NH_2), this basic moiety will act as a proton-acceptor in an acidic environment. It is then protonated forming a charged amide (-NH_3^+) side-group - rendering the whole molecule more amenable to microiontophoretic ejection and also more water soluble.

Establishing a potential difference between a drug solution and the external medium surrounding the barrel tip causes the movement of ions through the solution and results in drug ejection. By convention the direction of current flow is taken to indicate the flow of positive charge – outward and inward refer to the transport of positive charges out of or into a micropipette respectively. If an outward current is applied to a solution containing positively charged drug molecules (cations), the cationic (+) molecules will flow in the same direction as the current because of electrostatic forces. Alternatively if an inward current is applied to the same solution, cations will be retained in the barrel – anions (-) will move in the opposite direction.

“Barrel blocking” occurs when the resistance of the barrel is so high that the current generator cannot induce a flow of current at the available voltage. The barrel

resistance may be affected by the physical properties of the glass and tip orifice, the nature of the drug solution (such as solubility, molecular weight etc.) or by mechanical obstruction by dust or crystalline deposits. If current does not flow then no drug is ejected. “Barrel blocking” was more likely to occur if drug was ejected for a prolonged period.

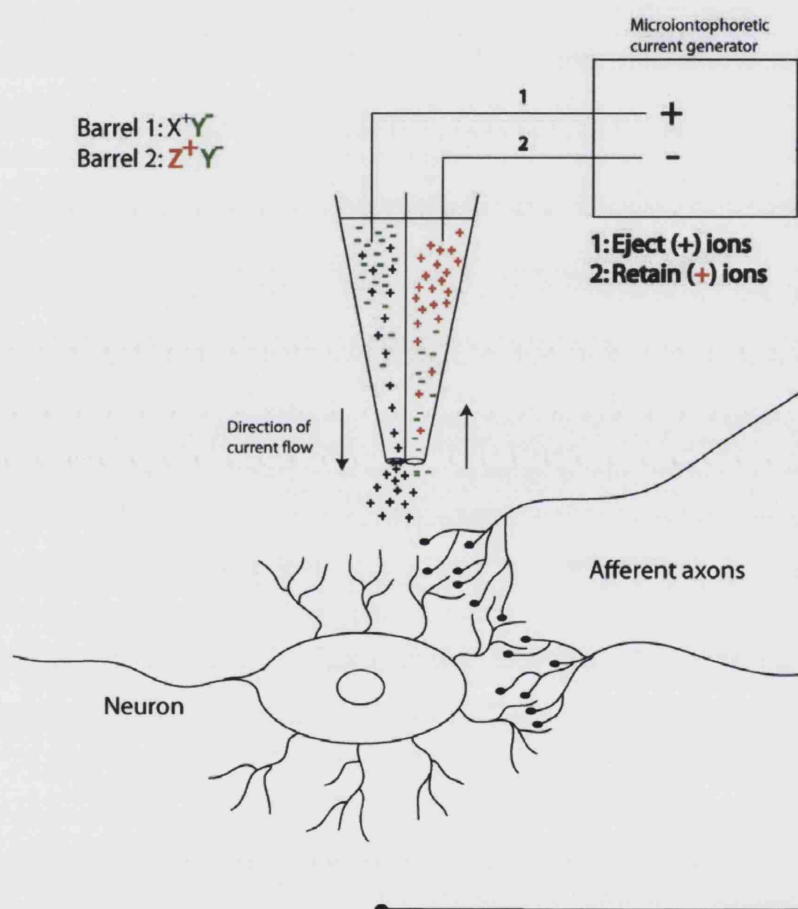


Figure 13: The principles of microiontophoresis

Two micropipette barrels are shown, each containing an ionic solution. The tips of both barrels are in close proximity to the cell body of the neuron under study. In barrel 1, X⁺ ions are being ejected by the outward flow of current (10-100nA) (current is by convention taken to mean the flow of (+) charge), while Y⁻ anions are retained. In barrel 2, Z⁺ ions are retained by a smaller (-5 nA) retaining current flowing in the opposite direction. This retaining current however results in the ejection of Y⁻ anions from the barrel. It is assumed that this has a negligible biological effect.

Current balancing and controls

Even when drugs are not being intentionally ejected, they may “leak” from a micropipette tip. This is because all molecules have a tendency to diffuse down a concentration gradient. Drug efflux will naturally occur from the tip of the micropipette into the surrounding environment. This leakage is minimised by using small retaining currents. Though these currents will retain the desired molecule, they will result in the ejection of oppositely charged ions from the barrel. As individual retaining currents are small (no more than 5-10nA) it is assumed that this ejection has minimal biological effects.

When multi-barrelled pipettes are used however, neurons are exposed to retaining and ejecting currents from several barrels. The sum of these currents may influence the excitability of the neuron. In an attempt to counter this, a barrel containing 1.0M NaCl is used for “current balancing”. A balancing current is continuously passed through this barrel sufficient to cancel out the sum of the ejecting and retaining currents passing through all the drug-filled micropipettes at any instant (Fig. 12).

Even when current balancing is employed, ejection of current may directly influence neuronal excitability. A control is often used to estimate the effect of current ejection. This contains a suitable ionic solution – in these experiments 0.2M NaCl or 0.05M sodium acetate. This is ejected with a current of the same direction and magnitude as the drug under study. It is also ejected for approximately the same duration. Just as the pH of the drug solutions may be adjusted by the addition of H^+ or OH^- ions (and these will also be ejected with the drug) so too is the pH of the control. Ejection of H^+ or OH^- ions may directly affect neuronal excitability so this must also be taken into account in any studies.

Difficulties in quantifying the effects of microiontophoretic drug ejection

The number of ions ejected from a solution in a micropipette is proportionate to the charge flux through the solution. In its most simple form, the molar flux (Q) of an ion produced by an ejection current (I) is:

$$Q = It / FZ$$

F: Faraday constant

Z: valency of the ion

t: transport number

Several factors influence the molar flux including the molecular weight (M) of the ion. The total flux of ions produced by a given quantity of charge is proportionate to M – as the molecular weight of a molecule increases, less will be ejected per unit of charge. The transport number (t) of any particular ion in a solution is the fraction of the applied current carried by that ion. Unfortunately the value of t for a drug may vary dramatically between micropipettes and even different barrels of the same multi-barrelled pipette. It is affected by a diverse range of factors including: the age of the micropipette, the pH of the solution, the concentration of small ions (such as Na^+ and Cl^-) in the solution, electrostatic interactions between the ion and the glass wall of the pipette and the nature of the receiving medium. The value of t can only be estimated for a particular compound and even if it can be given a value, calculating the molar flux is further complicated by drug ejection by non-microiontophoretic means. These include drug ejection by electro-osmosis and hydration effects.

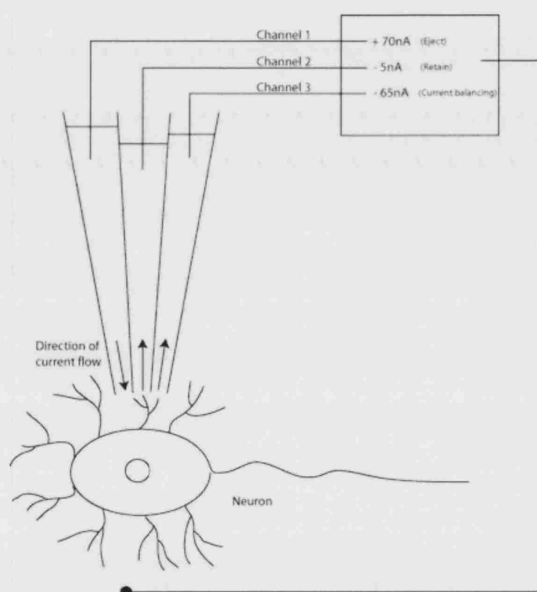


Figure 14: Microiontophoretic “current balancing”

The principle of “current balancing”. In this schematic diagram barrels 1 and 2 are being used for drug ejection and barrel 3 for current balancing. The direction and magnitude of the current in barrel 3 (-65 nA) is adjusted automatically to counter the net current flowing from the other barrels (+65 nA). Theoretically the neuron under study should not be influenced by current flow.

Drug ejection by electro-osmosis and hydration effects

Electro-osmosis is essentially the opposite of microiontophoresis. When an aqueous solution is in contact with a glass surface anions are tightly adsorbed onto the surface of the glass. A fixed layer of negative charge - an electrical “double layer” – is formed on the glass surface leaving the bulk of the fluid volume carrying a positive charge. The passage of current therefore causes the flow of the fluid volume in the direction of the current. The dissolved compound (especially if (+) charged) then moves with the fluid. This obviously favours compounds that are positively charged – it may even counter microiontophoretic ejection of anions. Electro-osmosis may contribute significantly to drug ejection from a micropipette – especially in dilute solutions or when the drug is poorly ionised (Fig. 13).

Hydration effects also influence the ejection of a compound from a micropipette. Water molecules are electrostatically attracted to any ion in aqueous solution. They form a hydration shell surrounding the ion. A drug – even one that is poorly ionised – which is soluble in water will be dissolved in this hydration cell. If an electric current flows through the solution the ion will move under its influence. As the ion migrates so to does its hydration shell - and any drug dissolved within it. Microiontophoresis in this case is only indirectly responsible for drug ejection (Fig. 13).

Drug diffusion after iontophoretic ejection

Unfortunately the electrical force that drives microiontophoretic ejection is limited to the micropipette barrel and the immediate environment of its tip. According to Ohm’s Law ($R = V / I$), the flow of electrical current (I) requires a potential difference (V) between two points in a circuit. As the electrical resistance (R) of the external medium – in this case nervous tissue - is generally substantially less than the internal environment of the micropipette, only a small potential gradient exists beyond the micropipette tip and current will therefore not flow. The tip may be considered to act as a point source of the drug and any further movement is dependent on diffusion. Diffusion constants differ between molecules and the conditions determining the extent of diffusion will differ between experiments – even for the same molecule. This introduces a further variable in any attempt to quantify the results of drug studies.

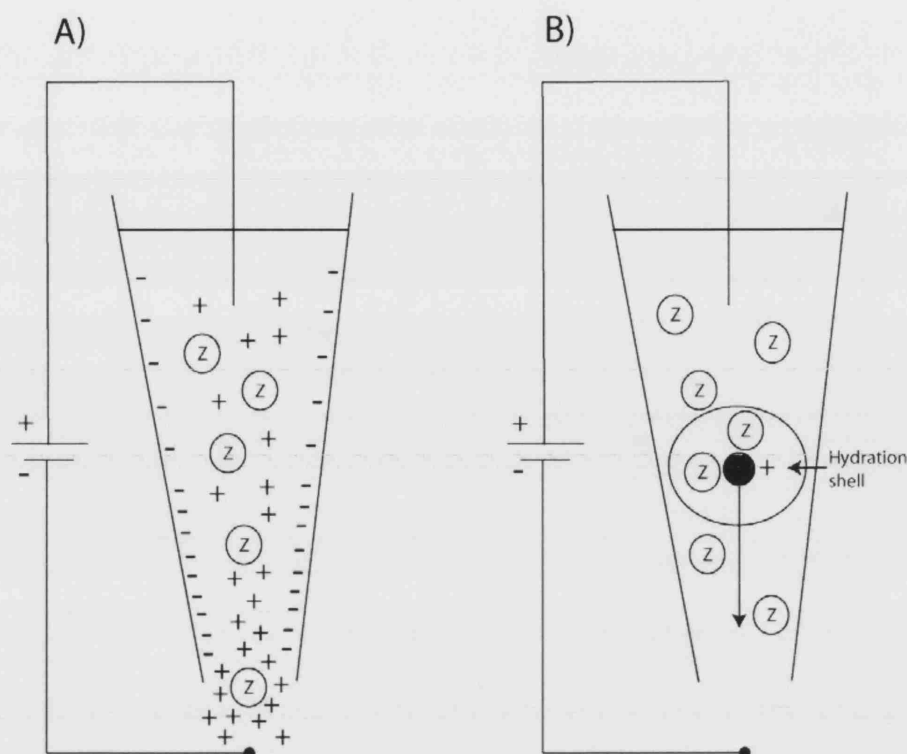


Figure 15: Electro-osmosis and hydration effects on drug ejection

Principles of drug ejection by electro-osmosis (A) and hydration (B). In electro-osmosis anions are adsorbed onto the surface of the glass micropipette. The solution containing the drug molecule Z has a net positive charge. The fluid then moves en-mass in the direction of the current (in this case outward) carrying molecules of Z with it. If Z is positively charged electro-osmosis will enhance its ejection – the opposite may occur if Z is an anion.

Hydration effects may also result in drug ejection. A shell of water molecules surrounds ions in solution. Molecules of a drug, Z, may become trapped in this shell and be ejected along with the ions. Adapted from (Stone, 1985).

The anatomical precision of drug delivery by microiontophoresis

As can be seen it is very difficult to quantify the amount of drug ejected by this technique. The concentration of drug that any given neuron is exposed to cannot be known. One partial exception is when a micropipette is used to study the effects of a drug on the same cell. In this case all variables should remain constant and the quantity of drug ejected will be proportionate to the ejecting current. Otherwise these experiments can provide only qualitative information concerning the receptors and ion channels involved in modulating trigeminovascular nociceptive neurotransmission. The great strength of microiontophoresis however is the anatomical precision that it provides in pharmacological studies. It allows drugs to be delivered to small numbers

of - or even individual - cells (Fig. 14). The relatively short length of the recording electrode tip - about 10 μm - has important electrophysiological properties. Short electrode tips are better able to discriminate the electrical activity of individual neurons. As it is unlikely that the ejected drugs diffuse over large distances, it can be assumed that the drug is present in a confined area and that the electrode is recording electrical activity from this same area. It is reasonable to assume therefore that any alteration in the excitability of a neuron is due to the effects of the ejected drug on that cell. When drugs are administered systemically (such as intravenously or intrathecally) it is not possible to localise their effects. This is particularly a problem in thalamic studies, as the drugs may have actions at more distal sites, such as the terminals of primary trigeminal nerves or in the trigeminocervical complex.

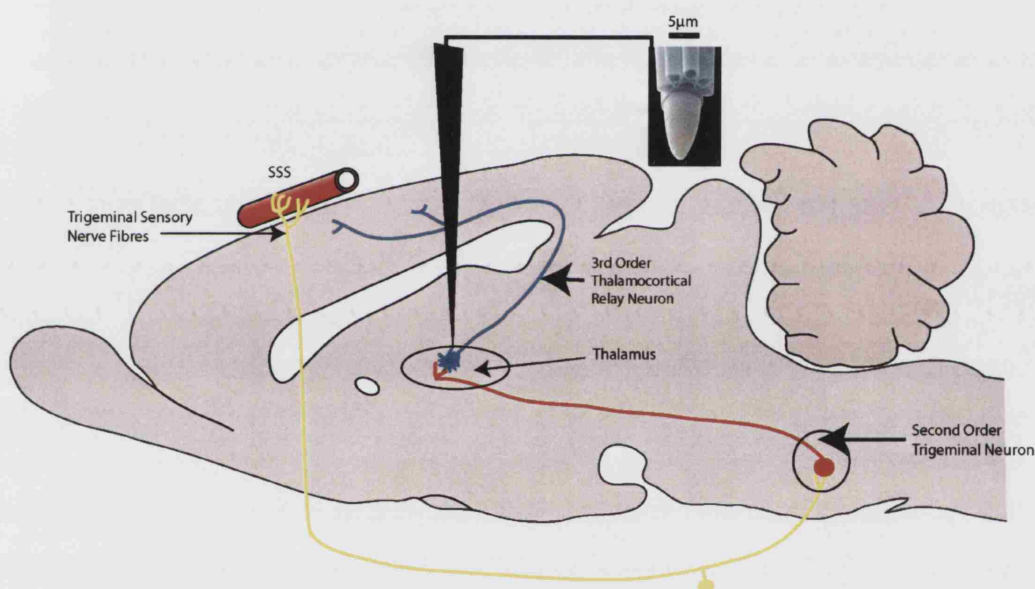


Figure 16: Electrophysiological recording from neurons in the VPM

Recordings were made from thalamocortical neurons in the VPM nucleus of the thalamus relaying trigeminovascular nociceptive information to the primary somatosensory cortex. Third order relay neurons were identified by electrical stimulation of the superior sagittal sinus (SSS). Ejection of L-glutamate was then used to identify the cell bodies of thalamocortical neurons. Recordings are made using carbon fibre electrodes. (The electron-micrograph was a gift from D. Budai, Kation Scientific, MN, USA)

Drugs used in microiontophoretic experiments

With the exception of Pontamine Sky Blue – which was dissolved in 0.1M sodium acetate (pH 6.5) – all drugs were dissolved in sterile de-ionised water. The pH was adjusted by adding small amounts of either 0.01M NaOH or 0.01M HCl to the solutions. As a result molecules were ionised - making them more amenable to microiontophoretic ejection and in some cases also improving their solubility. The drugs used in the three thalamic studies are listed in Tables 10-13.

Table 10: Drugs used in all microiontophoretic studies

Drug	Concentration	pH	Ejection Polarity	Source
L-Glutamate monosodium	0.2 M	8.0	(-)	Sigma Ltd.
NaCl	1.0 M	7.4	(+/-) Automated Current Balancing	Sigma Ltd.
Pontamine Sky Blue	2.5% (w/v)	6.5	(-)	BDH Laboratory Supplies

Sigma Ltd., St. Louis, MO, U.S.A.

BDH Laboratory Supplies, Poole, UK

(+): cation (-): anion

A microiontophoresis current generator (Dagan 6400, Dagan Corporation, MN, U.S.A.) provided ejecting and retaining currents for each test substance. Barrels were connected to the current generator head-stage by individual Teflon® coated silver wires (0.25 mm, WPI, USA). The distal end of the wire – which was placed in the drug solution – was stripped of its terminal 1cm of coating. Care had to be taken to avoid damage to the Teflon® coat while inserting wires into the barrels. Even though these wires were insulated, it was preferable that they did not touch each other, or any other part of the apparatus to avoid current switching artefacts. The head-stage was grounded to the stereotaxic frame.

One barrel was always filled with L-glutamate to identify and activate thalamocortical cell bodies - another with Pontamine Sky Blue to mark the recording site. One barrel was also filled with 1M NaCl for automated current balancing during all microiontophoretic experiments. Before each experiment the resistance of each barrel was measured; values of between 20 and 150 MΩ were typically recorded.

Table 11: Drugs used in Study 1

Drug	Concentration (M)	pH	Ejection Polarity	Source
GABA	0.2	4.5	(+)	RBI, Natrick, USA
(-)-Bicuculline methochloride	0.1	3.5	(+)	Tocris Cookson
2-OH Saclofen	0.1	9.0	(-)	Tocris Cookson
Muscimol	0.1	3.5	(+)	Tocris Cookson
(-)-Baclofen hydrochloride	0.1	3.0	(+)	Tocris Cookson
Sodium valproate	0.5	7.4	(-)	Sanofi-Synthelabo
NaCl*	0.2	7.4	(-/ +)	Sigma Ltd.
NaCl	0.2	3.5	(-/ +)	Sigma Ltd.
NaCl	0.2	9.0	(-/ +)	Sigma Ltd.

* Ejected as a control at comparable currents and for similar durations as the active drug

RBI, Natrick, MA, USA; Tocris Cookson, Avonmouth, UK; Sanofi-Synthelabo, Surrey, UK

Sigma Ltd., St. Louis, MO, U.S.A.

(+): cation (-): anion

Table 12: Drugs used in Study 2

Drug	Concentration (M)	pH	Ejection Polarity	Source
Naratriptan hydrochloride	0.025	5.5	(+)	Glaxo Wellcome Research and Development.
Ergometrine maleate	0.05	4.5	(+)	RBI, Natrick, USA and Tocris Cookson
(R)-(+)-8OH-DPAT	0.1	4.5	(+)	Tocris Cookson
GR127935 hydrochloride	0.02	4.0	(+)	Tocris Cookson
(S)-WAY 100135	0.01	4.5	(+)	Tocris Cookson
NaCl*	0.2	4.5	(+)	Sigma Ltd.

* Ejected as a control at comparable currents and for similar durations as the active drug

Glaxo Wellcome Research and Development, Herts., UK (+): cation (-): anion

Table 13: Drugs used in Study 3

Drug	Concentration (M)	pH	Ejection Polarity	Source
(S)-(-)-Propranolol	0.1	4.5	(+)	Tocris Cookson
(S)-(-)-Atenolol	0.025	4.5	(+)	Tocris Cookson
ICI118,551 hydrochloride	0.01	4.5	(+)	Tocris Cookson
SR59230A hydrochloride	0.05	4.5	(+)	Tocris Cookson
(-)-Isoproterenol hydrochloride	0.2	4.5	(+)	Tocris Cookson
Procaterol hydrochloride	0.1	4.5	(+)	Tocris Cookson
NaCl*	0.2	4.5	(+)	Sigma Ltd.

* Ejected as a control at comparable currents and for similar durations as the active drug

(+): cation (-): anion

Anions (-) (such as L-glutamate and valproate) were retained in their barrels with small positive currents (5nA) while cations (+) (such as naratriptan and propranolol) were retained with negative currents (-5nA). Ejection currents in directions opposite to the retaining currents were used, ranging from 10 to 110nA. As any effect of microiontophoretic drug ejection on the responses of third order relay cells to SSS stimulation or ejection of L-glutamate could be a current or pH artefact, all cells acted as their own controls. The control consisted of ejection of either Na⁺ or Cl⁻ ions - matching the polarity of the ejected drug - and also H⁺ or OH⁻ ions in those cases where pH alterations had been made to the solutions (this was so in Studies 2 to 4, Study 1 differed in that the pH of the NaCl control was not adjusted; instead a series of studies were undertaken comparing the effects of saline at neutral, acidic and alkaline pHs on thalamocortical neurons). The control was ejected at the same current as the drug under investigation and also for approximately the same duration. During experiments studying the response of thalamic cells following electrical stimulation of the SSS both the drug and the control could be ejected for up to 6 minutes.

Analysis of the neuronal response to electrical stimulation of the SSS

To record the response of units following electrical stimulation of the SSS, post-stimulation histograms were constructed. Each histogram was constructed by calculating the number of spikes recorded per 1ms bin (with a total sweep length of 50ms) elicited by 50 electrical stimuli. The main body of the response was found to occur with a latency of 10-20ms (Fig. 15). The mean number of spikes per bin in the main body of the response was calculated and converted to a percentage (%) by multiplying by two. The baseline response probability was calculated from up to three trials. A response probability of at least 35% was required for a cell to be considered responsive to trigeminovascular nociceptive stimulation and suitable for further study (Nagler et al., 1973).

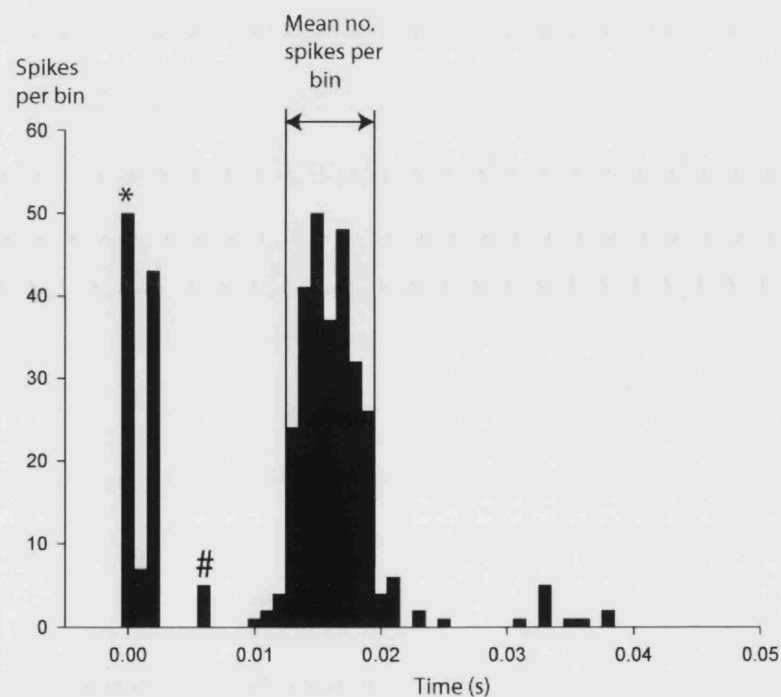


Figure 17: Post-stimulus histograms: calculation of response probability

Post-stimulus histogram demonstrating the response of a representative third order neuron in the VPM to electrical stimulation of the SSS. The number of spikes per 1ms bin was collected over 50 stimuli. The mean number of spikes per bin was then calculated – this was converted to a response probability (%) by multiplying by 2. (* stimulus artefact, # this short latency response (~5ms) was often observed and may represent activation of corticothalamic afferents)

Analysis of the response to L-glutamate ejection

Neuronal action potential firing in response to microiontophoresis of L-glutamate was analysed as cumulative rate histograms. The data was collected into successive one second bins (Fig. 16). The ejection current of L-glutamate was titrated for each cell to produce a sustainable firing rate - comparable to the response elicited by stimulation of the receptive field (Fig. 18). It was then intermittently ejected in trains of five second pulses on a 50% ejection/retention cycle. The effect of a given compound on the neuronal response to microiontophoretic ejection of L-glutamate was studied using cohorts of five successive pulses of L-glutamate. Five pulses were used as the firing rate could naturally vary between pulses – this was used to reduce the possibility of registering a spurious effect. The mean firing rate during each of the five second ejection periods was calculated using the “Spike®” software package (Cambridge Electronic Design, Cambridge, UK). The mean background firing rate – during the 5s retention phase – was calculated and subtracted from the firing rate during the preceding ejection phase to calculate the net effect of L-glutamate (Fig. 16).

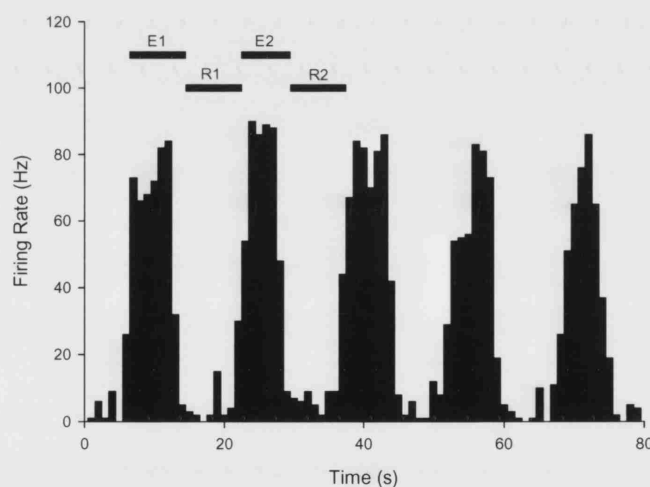


Figure 18: Peri-stimulus histograms: calculation of L-glutamate response

The mean firing rate of a thalamocortical neuron during each 5s ejection of L-glutamate was calculated by measuring the rate during the retention phase (R). This was subtracted from the firing rate during the corresponding 5s ejection phase (E) so that the increase in cell firing due to L-glutamate could be calculated.

General study design

The responses of cells to both stimuli were studied under three test conditions: a) baseline (i.e. no drug ejection) b) control and c) the drug under investigation. In experiments examining the modulation of the response to SSS stimulation the baseline response probability was calculated following 50 stimuli - no drug was ejected. This was repeated with the control and finally the active drug. Sufficient time was allowed to elapse between each stage to allow the cell to recover.

The baseline response in studies looking more specifically at “post-synaptic” drug actions was calculated from the firing rate during five second pulses of L-glutamate. The control and drug under investigation were in turn ejected with L-glutamate. L-glutamate was continuously ejected on its 50% ejection/retention cycle – even if a marked inhibition was observed. This allowed the neuron’s recovery to be monitored and ensuring that the effect was not due to the cell drifting away from the electrode tip. In the case of studies using antagonists, four test conditions existed: a) baseline, b) antagonist, c) drug and d) drug and antagonist (Fig. 17).

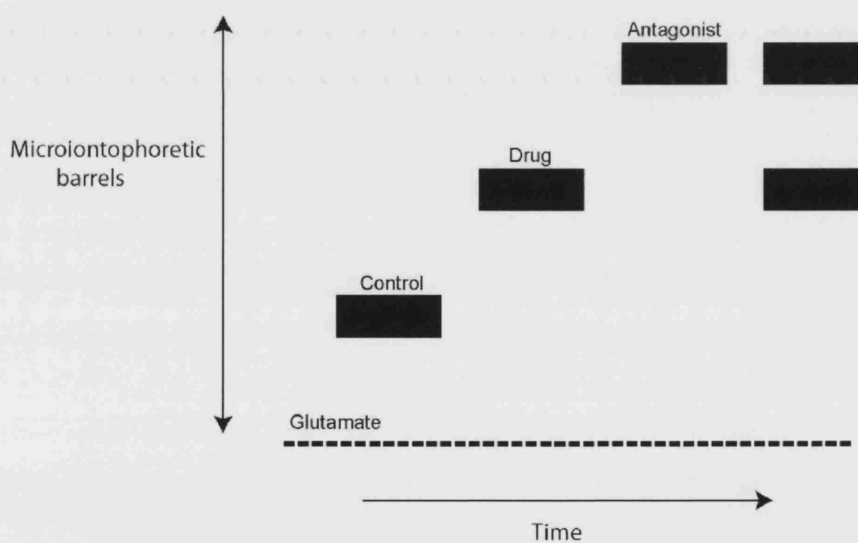


Figure 19: Drug ejection protocol

Diagram demonstrating schematically the protocol for drug ejection during microiontophoretic experiments. Due to restrictions on the number of available barrels not all combinations could be conducted on each individual cell.

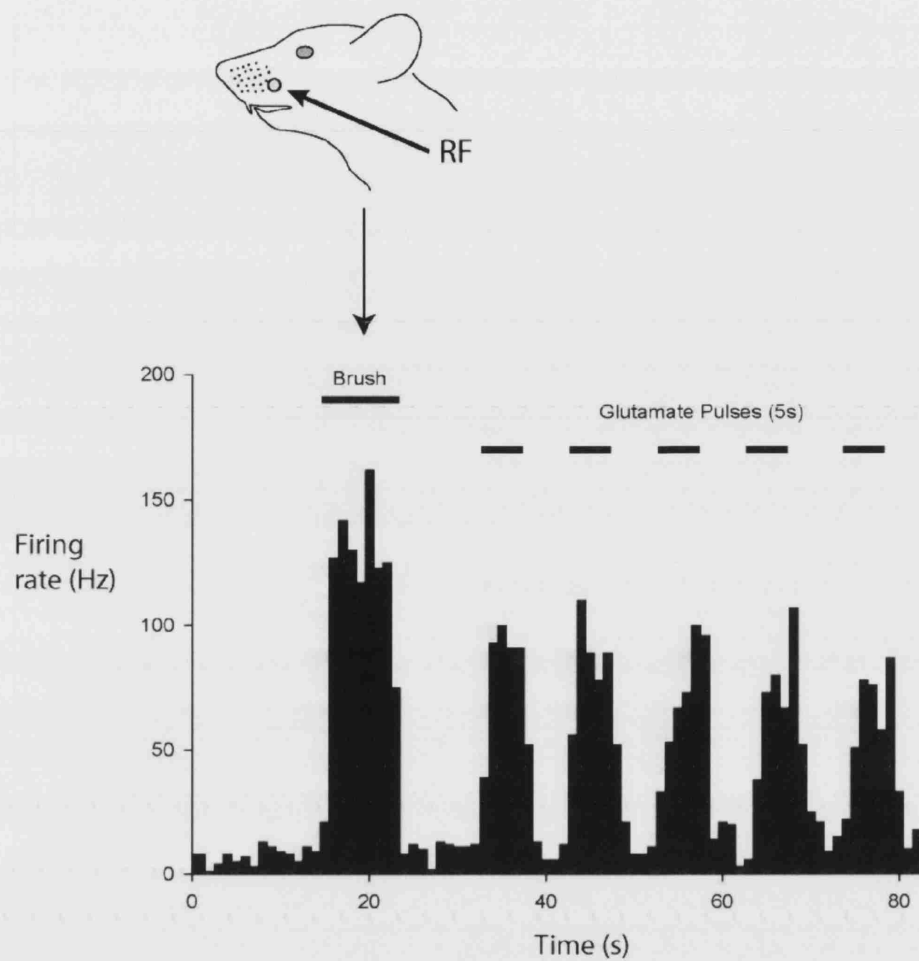


Figure 20: Titration of L-glutamate response

The ejection current of L-glutamate was titrated to generate a sustainable mean firing rate comparable to that following stimulation of the neuron's receptive field (RF) – in this case movement of a vibrissa.

Intravenous administration of naratriptan

In Study 2 the effects of intravenous doses of naratriptan on the responses following SSS stimulation were studied. This was done in an attempt to quantify the concentration of naratriptan achieved during microiontophoretic ejection. Naratriptan was dissolved in de-ionised sterile water to yield a 5mg/ml solution. A dose of 5mg/kg was used - equating to an injected volume of 1ml/kg body weight, an acceptable volume load for a 300g rat. The solution was filtered (0.02µm inorganic membrane filter) and given by a slow i.v. injection over one minute. The animal's haemodynamic parameters, blood pressure and heart rate, were monitored throughout. Post-stimulus histograms were recorded at 5 and 10 minutes and then every 10 minutes for up to 1 hour following the injection.

Histological confirmation of recording sites

The location of each recording site was verified by two methods. The first involved direct marking of the recording site by ejection of Pontamine Sky Blue. A microiontophoretic pump (BAB-350 Iontophoresis Pump, Kation Scientific, Minneapolis, USA) was used to eject Pontamine using a current of 2µA for 10 minutes. Alternatively the depth of each recording site was recorded from the microdrive and two reference points were then marked along the electrode tract dorsal and ventral to the recording sites. The recording sites were then reconstructed using these reference points. At the end of each experiment the brain was removed and placed immediately in neutral buffered 10% formalin (Sigma Ltd.) for 24 hours. This was slowly replaced by 30% sucrose (BDH Laboratory Supplies, UK) solution over subsequent days. Once the brain had fully equilibrated in 30% sucrose it was removed and a 5mm thick coronal section encompassing the thalamus and electrode tract was removed. This tissue block was cut using a freezing microtome into 40µm sections and placed on microscope slides. Sections were then examined (Axioplan microscope, Zeiss, Germany at 2.5x10 magnification) to locate Pontamine spots. Those sections in which blue marks were found were stained with neutral red and photographed (AxioCam MRc5 microscope camera, Zeiss, Germany). Recording sites were then reconstructed, approximating their original positions into two suitable coronal planes for diagrammatic purposes (Fig. 19).

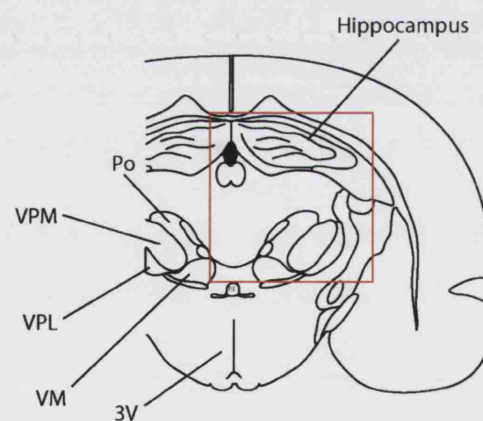
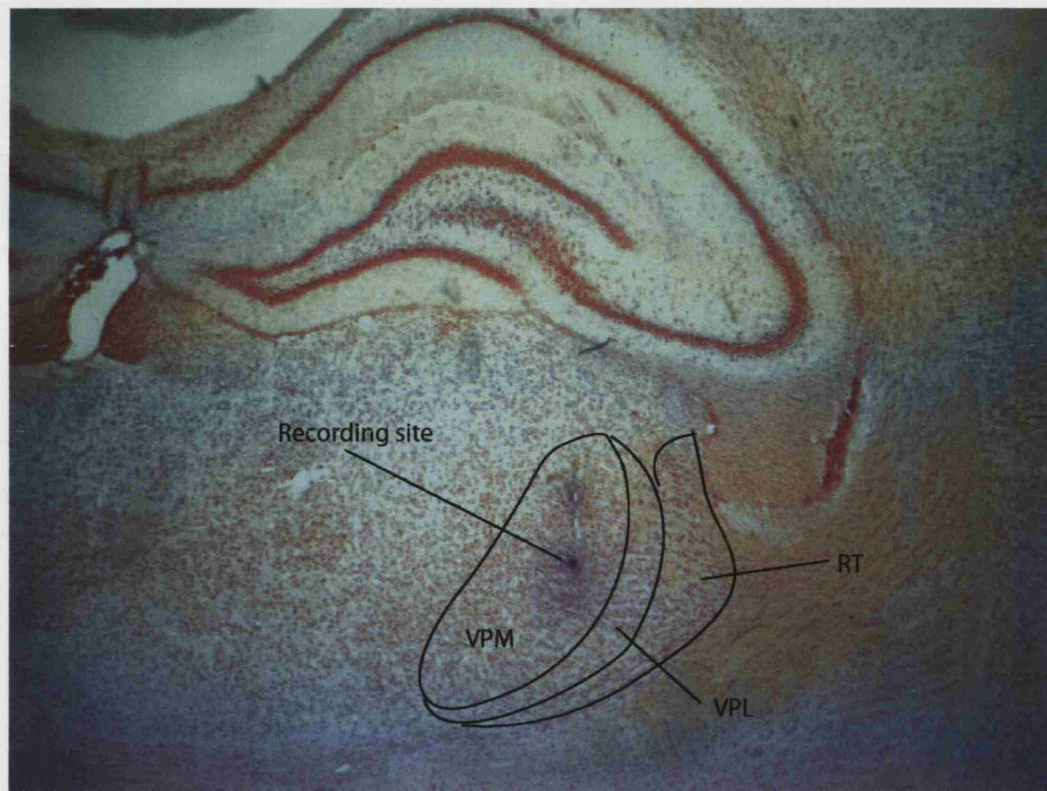


Figure 21: Histological confirmation of VPM recording sites

The location of electrode recording sites were confirmed histologically by either directly marking the site (as is the case in the top diagram) by ejection of Pontamine, or reconstructed from marked reference points. The bottom diagram indicates the field of view of the photomicrograph. Adapted from (Paxinos and Watson, 1986).

(VPM: ventroposteromedial nucleus, VPL: ventroposterolateral nucleus, RT: thalamic reticular nucleus, Po: posterior complex, VM: ventromedial nucleus, 3V: third ventricle).

Selection criteria used to identify cells suitable for microiontophoretic study

Thalamocortical neurons were required to display the following criteria before they were selected for microiontophoretic study (Fig. 20):

a) Electrical stimulation of the SSS was the primary method used to identify potential thalamocortical neurons. Neurons had to respond to electrical stimulation of the SSS with a response probability of at least 35% before further characterisation was undertaken. This minimum required level of response ensured that only neurons activated by a nociceptive trigeminovascular input were studied (Nagler et al., 1973).

b) Once a suitable unit was identified on the basis of its response to electrical stimulation of the SSS, L-glutamate was ejected. As glutamate receptors are predominantly located on the cell body of neurons, an increased firing rate in response to L-glutamate ejection was taken as evidence that the electrode was recording from the somata of a thalamocortical neuron and not from an axon. As intrinsic interneurons are scarce in the VPM it is reasonable to assume that any increase in firing rate following L-glutamate ejection truly reflects activity of third order relay cells. The ejection current of L-glutamate was titrated to produce a sustainable and reproducible firing rate. This was comparable to the firing rate triggered by stimulation of the cell's cutaneous receptive field (Fig. 18).

c) Each cell was required to have a cranio-facial cutaneous receptive field (RF). The neurons studied in these experiments were therefore activated by convergent viscerosomatic inputs from dural vascular and cutaneous structures. Modulation of the cutaneous inputs however was not studied.

The number of cells studied per animal varied ranging from 1 up to a maximum of 5, though typically 2 to 3 cells were recorded.

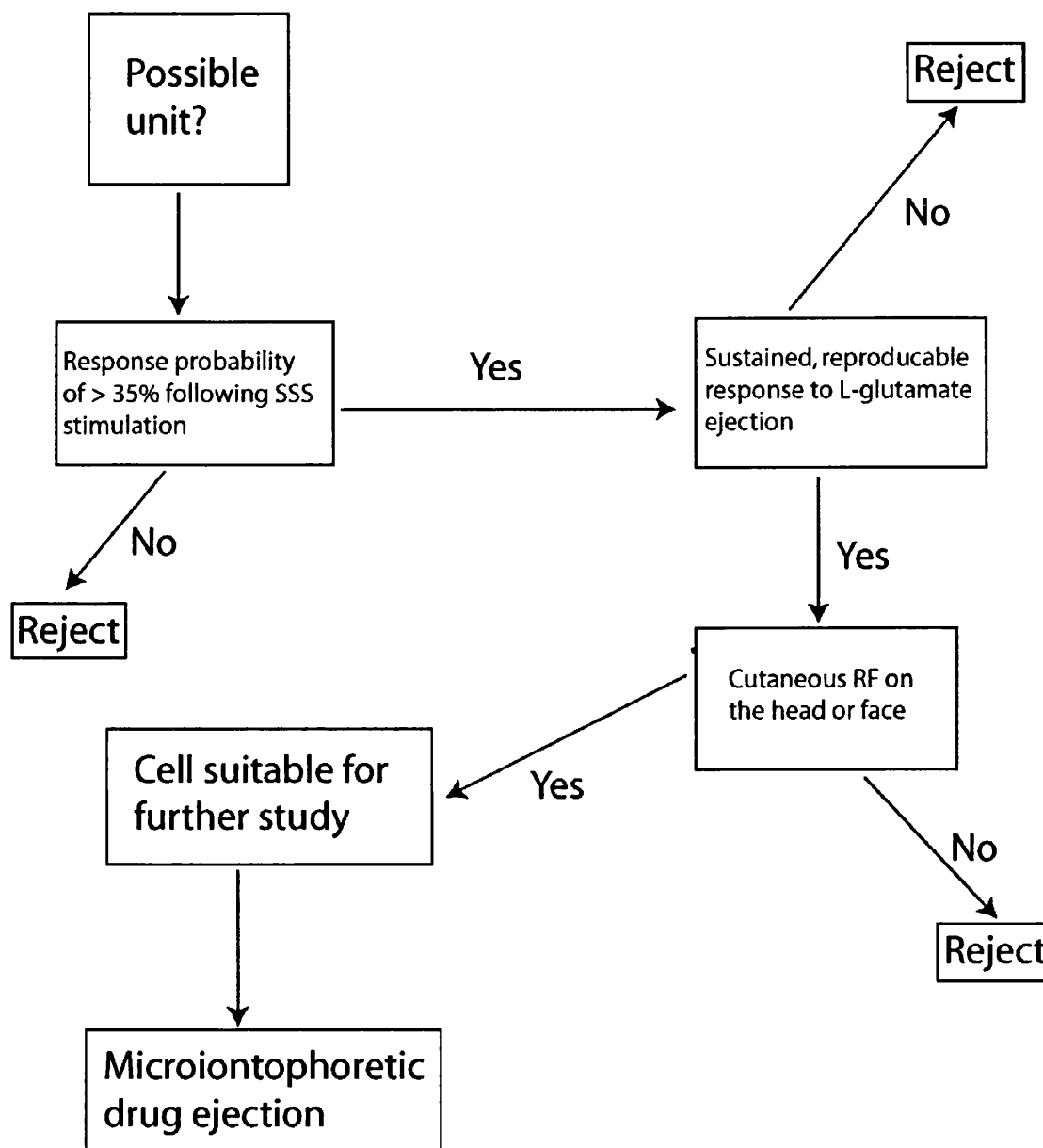


Figure 22: Algorithm for selecting neurons for microiontophoretic studies

(RF: receptive field)

Statistical Analysis

The results from the two types of experiment were analysed using different methods - both were performed using SPSS v 11.5 (SPSS, Chicago, USA).

Electrical stimulation of the SSS

The response probability of neurons following electrical stimulation of the SSS was calculated for each cell under the test conditions described. The results from all cells were analysed together and compared by a series of student's paired value *t*-tests to examine the effect of each intervention in turn (Table 14). This approach was taken to make sure that any artefact caused by current ejection was not mistaken for a drug effect. Significance was assessed at the $P < 0.05$ level. The mean response probability and the standard error of the mean (SEM) for each treatment group was also calculated.

Table 14: Student's paired *t*-test comparisons performed to study the effects of drugs on the responses of neurons following SSS stimulation

Drug Studies	Drug /Antagonist Studies
Control vs. Baseline	Drug vs. Baseline
Drug vs. Baseline	Antagonist vs. Baseline
Drug vs. Control	Drug + Antagonist vs. Baseline
	Drug vs. Drug + Antagonist

This framework of paired analyses was also used for studies looking at the effects of drugs on the L-glutamate evoked "post-synaptic" cell firing (see below)

Cell firing evoked by L-glutamate ejection

The mean net firing rate was calculated for each of five successive pulses of L-glutamate during the above test conditions. Repeated measures ANOVAs were computed with two factors: Drugs and Repeats, to determine intra- and inter- drug effects and interactions. Bonferroni corrections were applied and post-hoc comparisons with a Student's paired *t*-tests were performed. When the assumption of sphericity with regards to the factor of Repeats was violated, adjustments were made for the degrees of freedom and *P* values according to the Greenhouse-Geisser correction. As with the experiments examining the response following SSS stimulation, we analysed the effects of each intervention on the response to L-glutamate in turn. *F* and *P* values comparing the effects of each intervention will be

cited. Results were finally pooled and the mean response during each test condition was calculated (\pm SEM) for each of the treatment groups (Fig. 21).

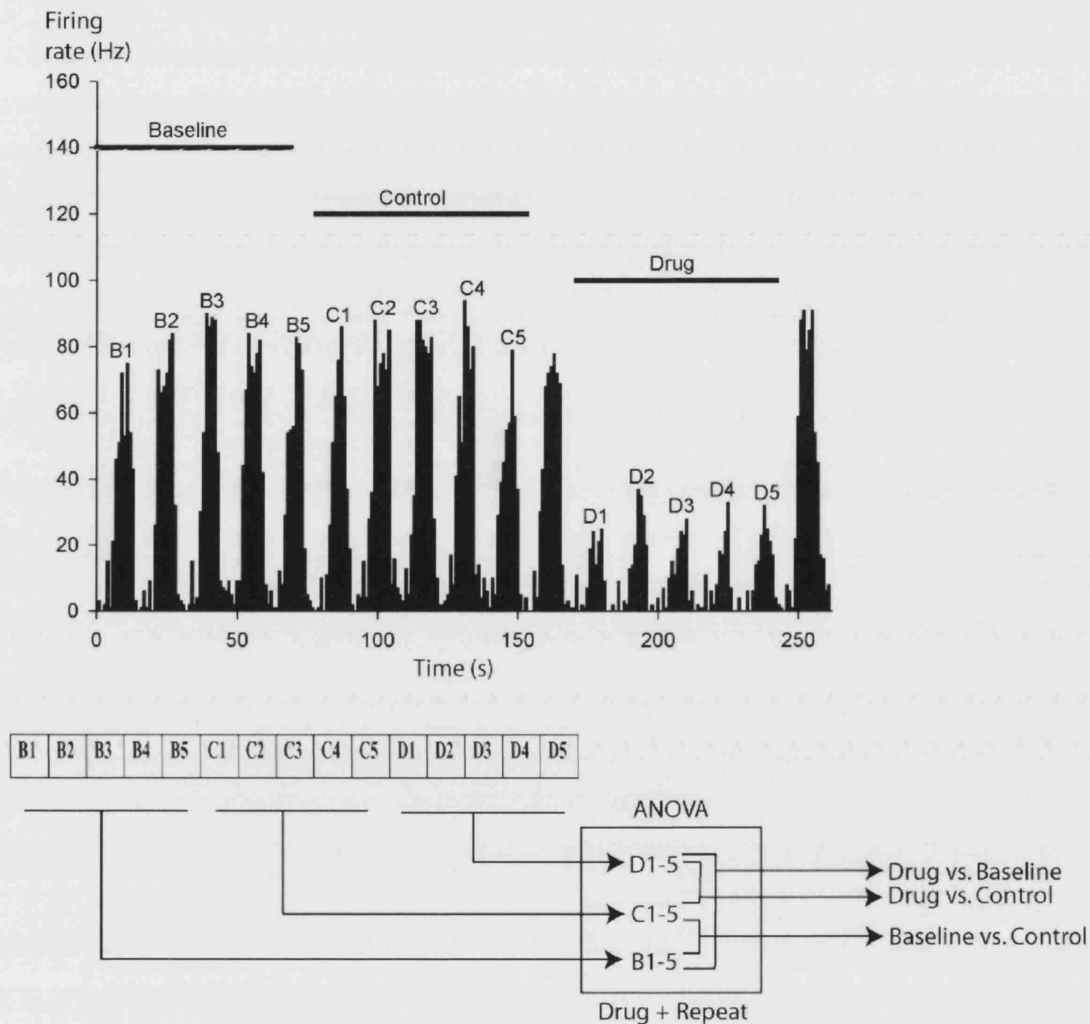


Figure 23: L-glutamate evoked response: analysis

Analysis of the effects of co-ejection of drug and control on L-glutamate evoked neuronal firing. The mean net firing rate was measured for each pulse in a cohort of five pulses of L-glutamate. As can be seen, the response to L-glutamate ejection could naturally vary within a cohort. Analysing five pulses reduced the risk of spuriously detecting a drug effect. Repeated measures ANOVAs were calculated comparing the effects of Drug and Repeats.

Cat experiments

In the following description, unless otherwise stated, the methods used were the same as in the preceding discussion on rat experiments.

Anaesthesia

Anaesthesia was induced with intraperitoneal, warmed (38°C) α -chloralose (60mg/kg) (Sigma) dissolved in sterile water. During surgery anaesthesia was maintained by inhalation of halothane (Rhodia Organique Fine Ltd., Bristol, UK) (0.5 – 3%) with a mixture of medical air (1.2 L/min) and oxygen (0.4 L/min) (BOC, UK). Once surgery was completed the volatile gaseous anaesthetic was discontinued and anaesthesia was maintained using intravenous α -chloralose in 2-hydroxypropyl- β -cyclodextrin (RBI, USA), given intravenously at a rate of 5 – 10 mg/kg/hr. At the end of each experiment cats received a lethal bolus of pentobarbitone sodium (Lethobarb[®], 200mg/ml – 3ml).

Preparation for physiological monitoring

A left inguinal incision was made to expose the left femoral neurovascular bundle. The femoral artery and vein were separated and cannulated with bespoke central lines (4F, Simms Portex Ltd., Kent, UK). Both were advanced into the great vessels near the heart – patency was confirmed by free back-flow of blood and lines were flushed with 0.9% saline and heparin sulphate (10 iu/ml, Multiparin[®], CP Pharmaceuticals, UK). The arterial line was connected to a pressure transducer (Ohmeda DTX Plus pressure transducer with PM-1000 transducer amplifier from CWE Inc.) for blood pressure monitoring. The venous line was used for fluid and drug administration.

The trachea was intubated with an endotracheal tube (Simms Portex, UK - 6.2 mm outer diameter) following topical anaesthesia of the larynx with lidocaine hydrochloride (Intubeaze[®], Shrewsbury, UK). Symmetrical incisions were then made on either side of the neck at the level of the first cervical vertebra (C₁). The transverse processes of C₁ were exposed by blunt dissection through the neck musculature, stripped of their muscular insertions and packed with gauze dressings.

The cat was then mounted in a stereotaxic frame (Kopf Instruments, USA) - the ear-bars were pre-treated with a local anaesthetic cream (Emla Cream[®], Astra Zeneca, UK) before insertion. The frame rested on an air table (TMC, Peabody, MA, USA) and during electrophysiological recording sessions this was supported by a cushion of

nitrogen gas. The intention was to reduce vibration artefacts originating from the environment during experiments.

The urinary bladder was catheterised with a Jackson/Foley urethral catheter (4F Simms Portex, UK) to monitor urine output and the corneas were covered with ointment (Lacrilube®, Allergan, Bucks., UK) to prevent drying. A rectal probe was used to monitor core body temperature, which was maintained between 37°C and 39°C by a heater blanket system (Harvard Apparatus, Holliston, MA, USA).

After neuromuscular blockade with gallamine triethiodide (Concord Pharmaceuticals, Essex, UK; initially 10 – 15 mg/kg i.v., maintenance 5 – 10 mg/kg/h) the subject was ventilated (665 Small animal ventilator, Harvard Instruments, USA) with a mixture of medical air (1.2 L/min) and oxygen (0.4 L/min) (BOC, UK). Periodically the depth of anaesthesia was assessed following withdrawal of neuromuscular blockade by testing for sympathetic and withdrawal responses following noxious stimulation. End-expired pCO₂ was continuously monitored (Capstar-100, CWE, Ardmore, PA, USA) and maintained between 2% and 4.5%. Arterial blood samples were also taken to ensure that an appropriate acid/base balance for a cat under α -chloralose anaesthesia (pH \approx 7.35) was maintained. Fluid (0.9% saline (Baxter Healthcare, Norfolk, UK)) was given intravenously to replace insensible losses (20 ml/h).

Cervical laminectomy and preparation of the spinal cord

A midline incision (6cm in length) was made on the dorsal aspect of the neck and the posterior neck muscles were dissected from the spinous processes and laminae of the first two cervical vertebrae. A partial laminectomy was performed on the C₁ and C₂ vertebrae with a dental drill (Volvere GX®, Nakanishi Dental, Japan). The site was irrigated continuously with 0.9% saline during drilling. The dura was exposed through a rectangular window (12 × 6 mm) straddling the two laminae. It was incised to create two flaps, which were reflected and fastened to the edges of the laminectomy with *N*-butylcyanoacrylate (Instant Super Glue®, ND Industries, Troy, USA). The spinal recording site was covered in saline soaked cotton-wool pellets until the microiontophoretic electrode was ready to be inserted into the cord. At that point the pia matter in the vicinity of the dorsal roots was incised gently and reflected using a sapphire tipped scalpel.

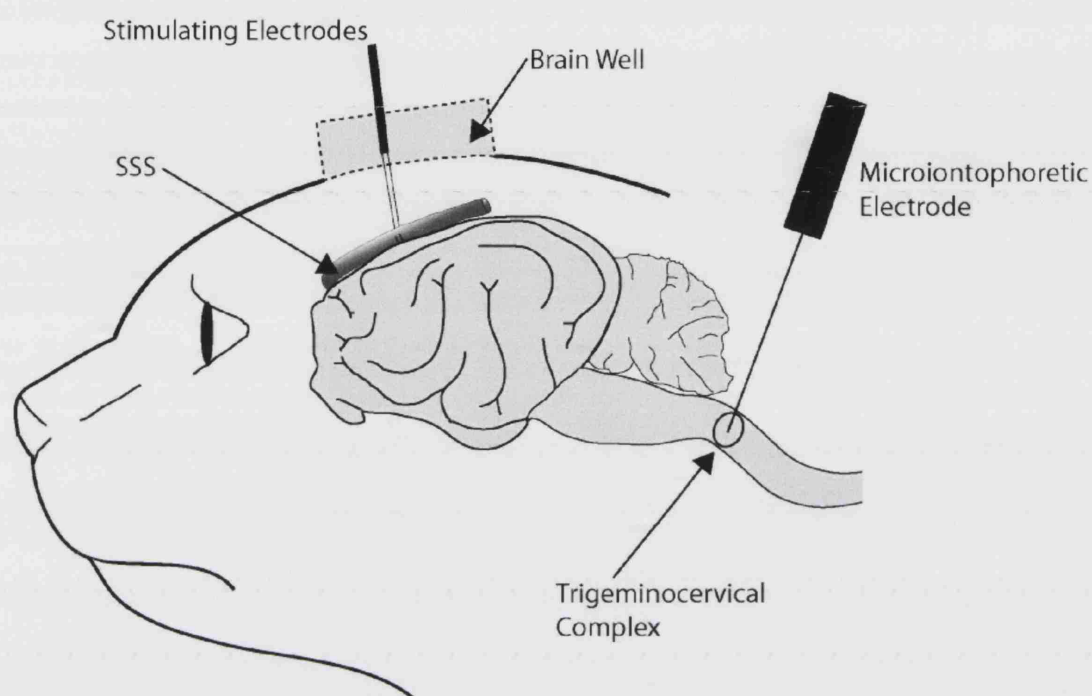


Figure 24: Surgical preparation of the cat

Diagrammatic representation of the felid preparation. The SSS was suspended on two platinum wire electrodes while neuronal recordings and drug ejection were performed using glass and tungsten multi-barrelled electrodes. The electrode was lowered into the trigeminocervical complex through a C₁-C₂ laminectomy. Neurons in the dorsal horn were identified by their responses following electrical stimulation of the superior sagittal sinus and ejection of L-glutamate.

Preparation of the craniotomy

A midline craniotomy was performed after the skin and aponeurosis over the cranial vault had been divided to expose the bone. This circular craniotomy, which had a diameter of approximately two centimetres, was centred on the vertex. The outline was drilled leaving a disc of bone. This was gently lifted off the dura to expose the SSS. Two parallel incisions were made in the dura either side of the sinus. The SSS was gently elevated and the falx cerebri was incised below it. A polyethylene sheet (5x20mm) was inserted under the sinus to isolate it electrically from the adjacent cortex. A polypropylene dam was fixed to the edges of the craniotomy with dental acrylic (Vertex, Zeist, Netherlands) and filled with liquid paraffin. The isolated SSS was carefully draped onto a pair of bipolar platinum hook electrodes connected to a stimulus isolation unit (S88, Grass instruments, USA) (Fig. 22).

Possible movement artefacts were minimised by clamping the transverse processes of C₁ bilaterally and fixing these to auxiliary ear bar holders on the stereotaxic frame. The remaining spinous process of C₂ and one of the thoracic vertebrae were also clamped to the frame. Bilateral pneumothoraces were performed to reduce respiratory movements by making intercostal incisions through the thoracic wall. They were kept patent with polythene tubes.

Electrophysiological recording

Extracellular recordings were made with a microiontophoretic combination electrode. These were fabricated in-house by incorporating a tungsten recording electrode (impedance 300 – 800 k Ω at 1 kHz in saline) into the central barrel of a seven-barrel glass multipipette (Stoelting, Wood Dale, IL, USA). Electrodes were pulled (Narashige Electrode Puller, Japan) and the tips etched to the desired dimensions: recording electrode tip length 10 μ m, width 5 μ m (Hellier et al., 1990). The same technique was used for filling the electrodes as in the thalamic experiments. The electrode was fitted to a piezoelectric microdrive (6000 ULN; Burleigh Instruments, NY, USA) mounted on a micromanipulator. Once the electrode was inserted, the exposed cord was covered in tissue grade agar (3% w/v in saline; Sigma, St. Louis, MO, USA) to minimise movement of the cord. The electrode was advanced into the region of the C₂ dorsal root entry zone in 5 μ m steps. As the microiontophoretic

electrode was advanced through the spinal dorsal horn supra-maximal electrical stimuli were delivered (square wave pulses, 120 – 140 V, 250 μ s, 0.3 Hz; S88 Stimulator, Grass instruments) to the SSS. This was used to activate primary trigeminal afferents just as in the thalamic studies. The signal was amplified, filtered and passed through a window discriminator using the same equipment and settings as in the thalamic studies. An exception was that data (physiological parameters and electrophysiological results) were saved on VHS magnetic tape (Sony, Japan) (Pulse Code Modulator; Vetter, Rebersburg, PA, USA).

Data collection and receptive fields

Post-stimulus histograms were constructed (bin width 1ms, sweep length 50 ms – 50 stimuli per histogram) on-line whenever a potential unit was identified. The response probability (%) of a unit was calculated with the same method used in the thalamic studies. If the unit had a response probability of > 35% it was regarded as being activated by trigeminovascular nociceptive afferents from the SSS (Nagler et al., 1973). Once a unit with a sufficient response probability was identified, L-glutamate was ejected to confirm that the recording was being made from the cell body of a second order neuron. If the cell responded to ejection of L-glutamate with a consistent and reproducible rate of firing, a search began for cutaneous receptive fields on the face or forepaws. These receptive fields were characterised using the same classification system - namely LTM, WDR and NS - as in the previous studies, though cells with only vibrissal inputs were not identified.

Drugs used in experiments and microiontophoresis

L-Glutamate monosodium, cadmium chloride and sodium chloride were dissolved in sterile de-ionised water. Sodium ions (Na^+) served as the control for cadmium ions (Cd^+). The high threshold VDCC peptide blockers were dissolved in 50 mM sodium acetate - this also served as the control for peptide ejection. Pontamine sky blue was dissolved in 0.1M sodium acetate solution. L-Glutamate monosodium 0.2M, NaCl 1.0M and Pontamine sky blue 2.5% (w/v) were used in all studies (Tables 15 and 16).

Table 15: Drugs used in all microiontophoretic studies

Drug	Concentration	pH	Ejection Polarity	Source
L-Glutamate monosodium	0.2 M	8.0	(-)	Sigma
NaCl	1.0 M	7.4	(+/-) Automated Current Balancing	Sigma
Pontamine Sky Blue	2.5% (w/v)	6.5	(-)	BDH Laboratory Supplies

(+): cation (-): anion

Table 16: Drugs used in Study 4

Drug	Concentration (mM)	pH	Ejection Polarity	Source
Cadmium chloride	200	7.4	(+)	Aldrich Ltd.
NaCl*	200	7.4	(+)	Sigma Ltd.
ω -agatoxin IVa	0.4	4.0	(+)	Peptide Institute, Osaka, Japan
ω -agatoxin TK	0.4	4.0	(+)	Peptide Institute, Osaka, Japan
ω -conotoxin GV1a	0.4	4.0	(+)	Peptide Institute, Osaka, Japan
calciseptine	0.4	4.0	(+)	Peptide Institute, Osaka, Japan
Sodium acetate [#]	50	4.0	(+)	BDH Laboratory Supplies

* Ejected as a control for Cd²⁺

[#] Ejected as a control for peptide VDCC blockers

(+): cation (-): anion

All peptides were ionised as cations and both they and cadmium ions were retained in the microiontophoretic barrels with negative currents (-5 nA). L-Glutamate and Pontamine sky blue were ionised as anions and retained with positive retaining currents of similar magnitude. Currents (20 – 100 nA) of opposite polarity to the

retaining currents were used for drug ejection. Microiontophoretic barrels had resistances of 40 – 150 M Ω .

General experimental protocol

Experiments followed the same protocol as thalamic microiontophoretic studies. Once a cell which was responsive to electrical stimulation of the SSS was found, L-glutamate was ejected to confirm that it was a cell body - and not an axon of passage. Its receptive field was defined and the experiment proceeded as already described with the exception that modulation of the response to SSS stimulation was not generally studied. As there are no antagonists for cadmium ions or peptide toxins, experiments were restricted to observing the effects of high threshold VDCC blockers on L-glutamate evoked neuronal firing. The mean neuronal firing rate for each one of the five second pulse of L-glutamate were measured. The results from five successive pulses of L-glutamate were then collected for the three test conditions: a) baseline, b) control and c) VDCC blocker.

Histological confirmation of recording sites

This was by either direct marking of the recording site or leaving Pontamine deposits as reference points of known depths. Sections were stained with neutral red and the recording sites were reconstructed for diagrammatic representation (Fig. 23).

Statistical Analysis

As already described repeated measures ANOVAs were performed to examine the factors Drugs and Repeats. Bonferroni corrections and post-hoc comparisons with a Student's paired *t*-test were performed. Where the assumption of sphericity with regards to the factor of Repeats was violated we made corrections for *P* values and degrees of freedom according to the Greenhouse-Geisser calculation. Significance was assessed at the $P < 0.05$ level.

Owing to the small numbers involved (ω -conotoxin GV1a: $n = 3$), the effect of VDCC blockers on superior sagittal sinus evoked responses was compared with a Friedman one-way ANOVA. *P* values were once again assessed at the 0.05 level of significance.

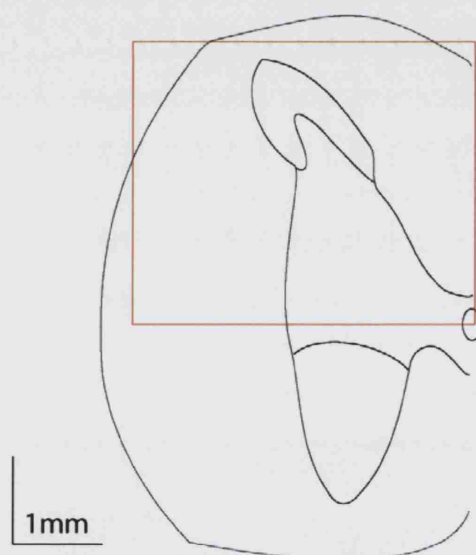
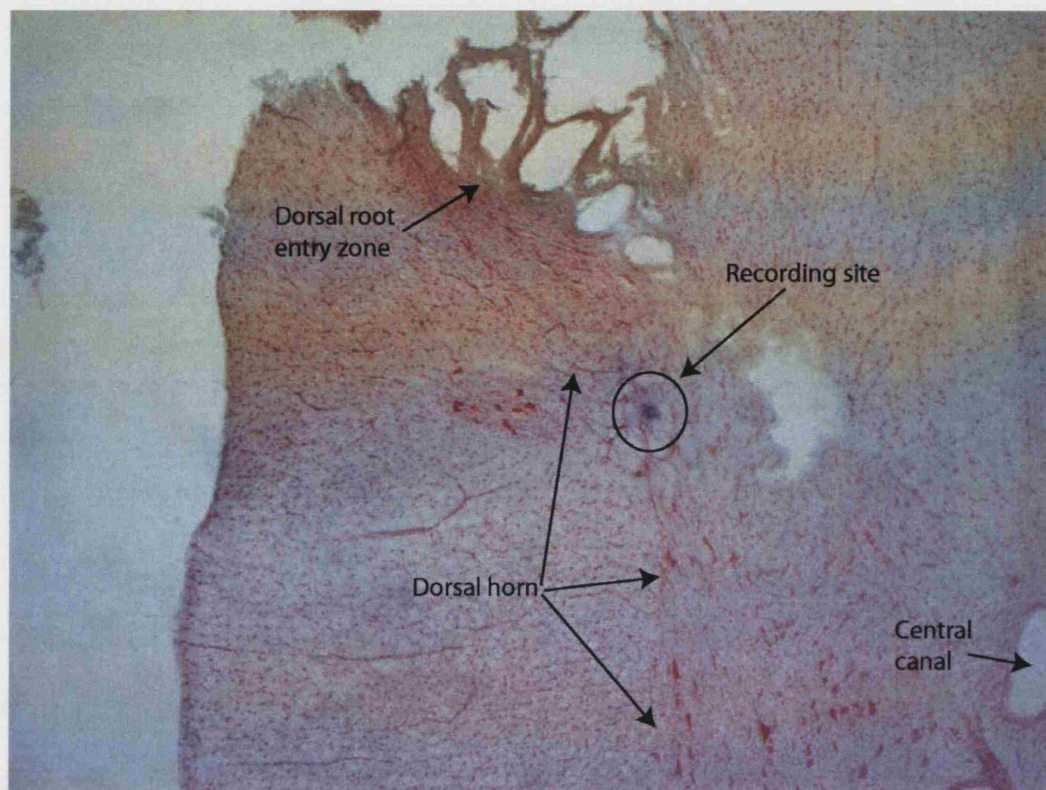


Figure 25: Histological confirmation of recording sites in the TCC

A typical recording site in the TCC marked by pontamine is shown in the upper diagram – the field of view is shown in the bottom schematic diagram.

Results

“Sweet wine is less likely to produce headache than is heavy wine, it has less effect upon the mind”

Hippocrates (460-375 BC)

Study 1: Investigation of the modulatory actions of γ -amino-butyric acid (GABA) and sodium valproate on trigeminovascular nociception by thalamocortical neurons of the rat VPM nucleus.

Introduction

The ventroposteromedial (VPM) nucleus is the principal thalamic relay for conveying the sensory discriminative aspects of nociceptive information to the primary somatosensory cortex (Steriade et al., 1997). Functional imaging studies suggest that activation of this region occurs in models of trigeminal pain and also during spontaneous attacks of migraine (Bahra et al., 2001; DaSilva et al., 2002). *In vivo* electrophysiological studies also demonstrate that trigeminovascular nociceptive stimulation activates neurons in several thalamic nuclei, notably the VPM nucleus (Angus-Leppan et al., 1995; Davis and Dostrovsky, 1988b; Zagami and Lambert, 1990). The modulation of nociceptive sensory transmission in the VPM is therefore of interest to migraine research.

One of the major modulatory inputs to the VPM arises from the thalamic reticular nucleus (RT) (Shosaku et al., 1989). GABAergic input from the RT not only modifies but also regulates the flow of sensory information to the cortex by adjusting the response properties of thalamocortical relay neurons.

GABA metabolism is a target for therapeutic interventions in migraine as several acute and prophylactic agents have GABAergic actions (Silberstein and Goadsby, 2002). Sodium valproate is a very widely used prophylactic treatment (Silberstein, 1996) though little is known of its site(s) of action. It can effectively block peripheral trigeminal activation (Cutrer et al., 1995; Lee et al., 1995) and may also act centrally, as demonstrated by its ability to modify abnormalities of cortical excitability in migraineurs using transcortical magnetic stimulation (Mulleners et al., 2002). Sodium valproate's efficacy in treating primary generalised epilepsy suggests that it may modulate thalamic activity (Shorvon, 2000), though whether this contributes to its actions in migraine is not known.

GABA modulates somatosensory transmission in various thalamic nuclei (Olausson et al., 1994; Reyes-Vazquez et al., 1986; Roberts et al., 1992; Salt, 1989; Yamashiro et al., 1994; Yamashiro et al., 1997). No studies however have specifically examined GABAergic modulation of trigeminal nociception. If GABA is capable of modulating trigeminal nociception in the thalamus, it may also be a site where putative GABAergic drugs act in the prophylactic treatment of migraine.

In this study *in vivo* electrophysiological recordings were made from the cell bodies of third order thalamocortical neurons relaying trigeminovascular nociceptive

information. We investigated whether ejection of GABA and sodium valproate could inhibit thalamocortical activity in response to superior sagittal sinus (SSS) stimulation. In addition we looked more specifically at “post-synaptic” actions, which in practice refers to neuronal elements including dendrites and cell bodies distal to the synaptic cleft, of these drugs through their modulatory actions on L-glutamate evoked third order neuronal firing. The pharmacology of GABA’s actions was dissected using selective GABA_A and GABA_B receptor agonists and antagonists (see Table 17).

As the pH of most drug solutions is adjusted by the addition of small amounts of H⁺ (or OH⁻) ions to aid in microiontophoretic ejection, the effect of pH was also specifically examined. Recording sites are shown in Fig. 39.

Table 17: Summary of drugs used in Study 1

Drug	Pharmacological Action	Ejection Polarity	Control
GABA	GABA agonist	(+)	Na ⁺ ions
Muscimol	GABA _A agonist	(+)	Na ⁺ ions
Baclofen	GABA _B agonist	(+)	Na ⁺ ions
Bicuculline	GABA _A antagonist	(+)	Na ⁺ ions
OH-Saclofen	GABA _B antagonist	(-)	Cl ⁻ ions
Sodium valproate	Possible GABAergic action	(-)	Cl ⁻ ions
NaCl (pH 3.5)	Control for pH effects (Na ⁺ /H ⁺ ions)	(+/-)	Na ⁺ /Cl ⁻ ions
NaCl (pH 9.0)	Control for pH effects (Cl ⁻ /OH ⁻ ions)	(+/-)	Na ⁺ /Cl ⁻ ions

(+): cation, (-): anion

When the effect of pH was specifically studied the pH corrected saline solutions were ejected using both (+) and (-) ejecting currents. In practical terms acidic solutions were invariably exposed to currents that ejected the drugs as cations, while in the case of alkaline solutions anions were typically ejected. The more relevant ions and ejection polarities are therefore in bold.

Results

A total of 73 cells were studied (see Table 18), each displaying convergent trigeminal viscerosomatic inputs. Cells responded with an increased probability of firing to electrical SSS stimulation with a probability of firing of $70 \pm 3\%$ (Mean \pm SEM). The results are presented in detail in Tables 19 to 22

Table 18: Receptive field characteristics of the neurons in Study 1

Receptive Field Characteristics	Number of cells
Low threshold mechanosensitive (LTM)	9
Nociceptive specific (NS)	1
Wide dynamic range (WDR)	28
Vibrissal (no. of vibrissae: mean \pm SEM)	35 (2.7 ± 0.2)

Effect of GABA and valproate on the response to SSS stimulation

Ejection of GABA inhibited the response following SSS stimulation in comparison to the control ($n = 13$, $P = 0.001$) in a reversible manner (Table 19, Fig. 24 and 25). The drug was ejected for between 1 to 4 minutes. Ejection of control had no effect in comparison with the baseline ($P = 0.7$). This inhibition could be reversed in all cells tested - though this number was small ($n = 3$, $P = 0.06$) - by simultaneous ejection of bicuculline.

Sodium valproate could also reversibly inhibit the response of thalamocortical cells to SSS stimulation ($n = 7$, $P = 0.001$). As with GABA ejection, valproate had to be ejected for between 1 to 4 minutes before a response was observed. The control (in this case ejection of Cl^-) again had no effect itself relative to the baseline response ($P = 0.9$) (Table 19, Fig. 24 and 26).

Table 19: Effects of GABAergic drug ejection on response probability following SSS stimulation

i) Mean response probabilities following SSS stimulation

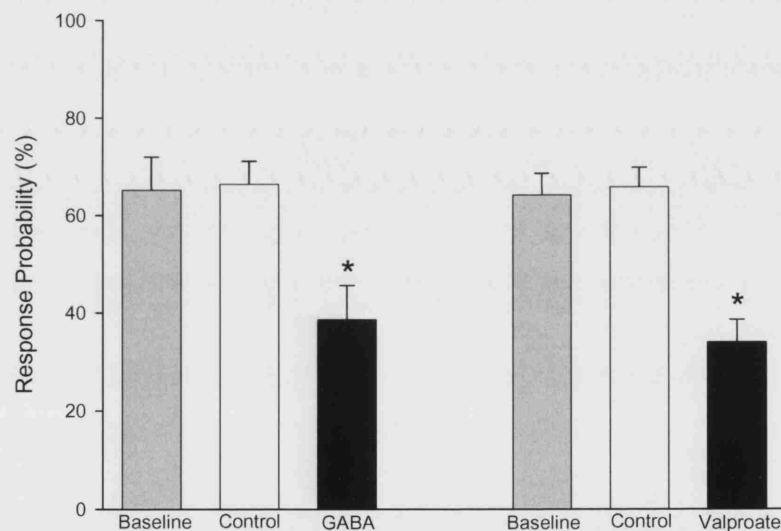
Drug	Baseline	Control (Na ⁺ , H ⁺)	GABA agonist
GABA	64 ± 4%	66 ± 4%	34 ± 5%*
Valproate	65 ± 8%	66 ± 9%	39 ± 7%*

Response probabilities of thalamocortical neurons following electrical stimulation of the SSS. When a neuron was located a series of 50 stimuli were delivered to the SSS and the response probability (as a percentage) calculated. Results under the three test conditions were pooled and are presented as the mean ± SEM (* $P < 0.05$).

ii) Statistical results

GABAergic agonist	Agonist v. baseline	Agonist v. control	Baseline v. control
GABA	$t_{12} = 0.4, P = 0.002$	$t_{12} = 4.4, P = 0.001$	$t_{12} = 0.4, P = 0.7$
Valproate	$t_6 = 8.6, P < 0.001$	$t_6 = 6.4, P = 0.001$	$t_6 = 0.2, P = 0.9$

Paired sample t -test analysis of the effects of GABAergic agonists on the response probabilities of thalamocortical neurons following electrical stimulation of the SSS.

**Figure 26: SSS stimulation: effects of GABA and valproate (pooled data)**

Comparison of the effects of GABA and valproate on the probability of thalamocortical neuronal firing in response to SSS stimulation. The control for GABA ejection involved ejection of Na⁺ ions and for valproate Cl⁻ ions. Mean (± SEM) response probabilities were calculated for all neurons tested (GABA $n = 13$, Valproate $n = 7$; * $P < 0.05$).

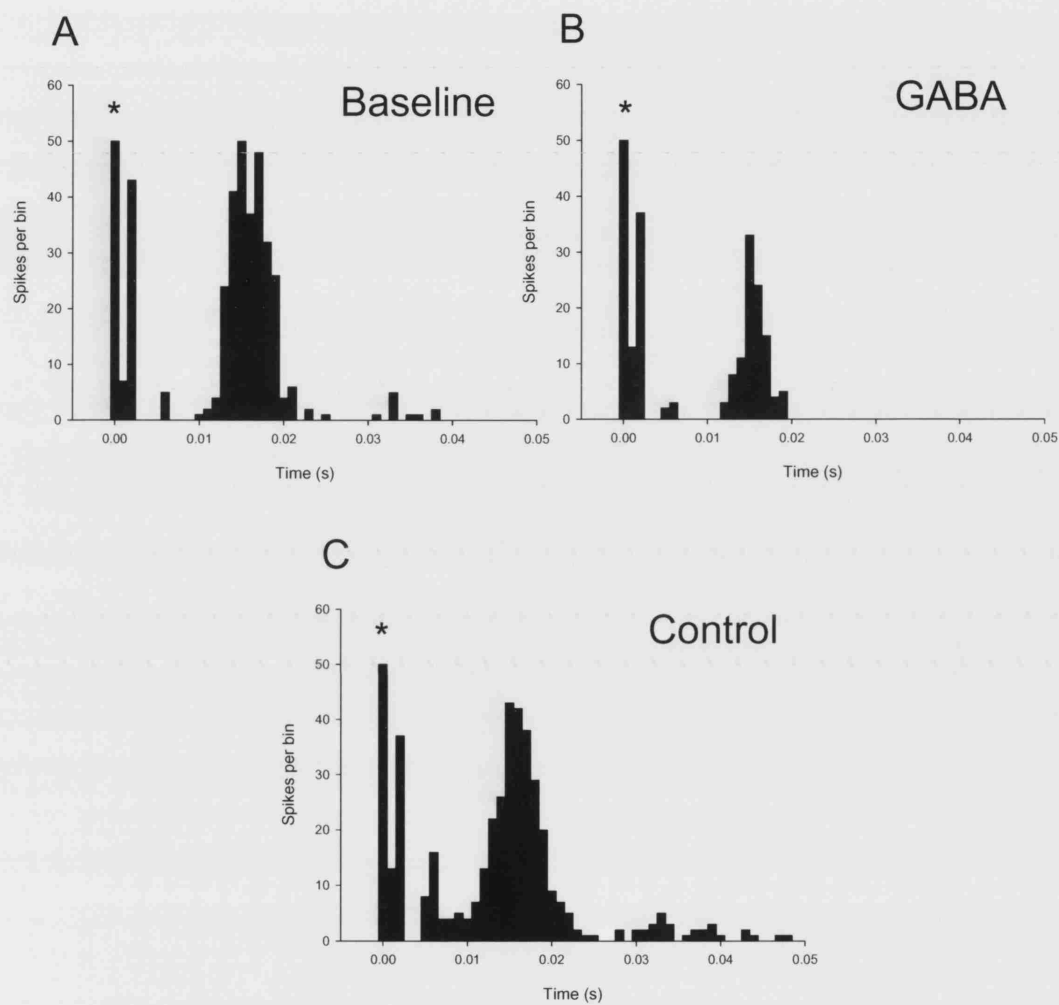


Figure 27: SSS stimulation: effect of GABA

A representative post-stimulus histogram obtained from a thalamocortical neuron following electrical stimulation of the SSS. A comparison of the effects of GABA, control (Na^+ ions) and the baseline response is shown.

(* Stimulus artefact)

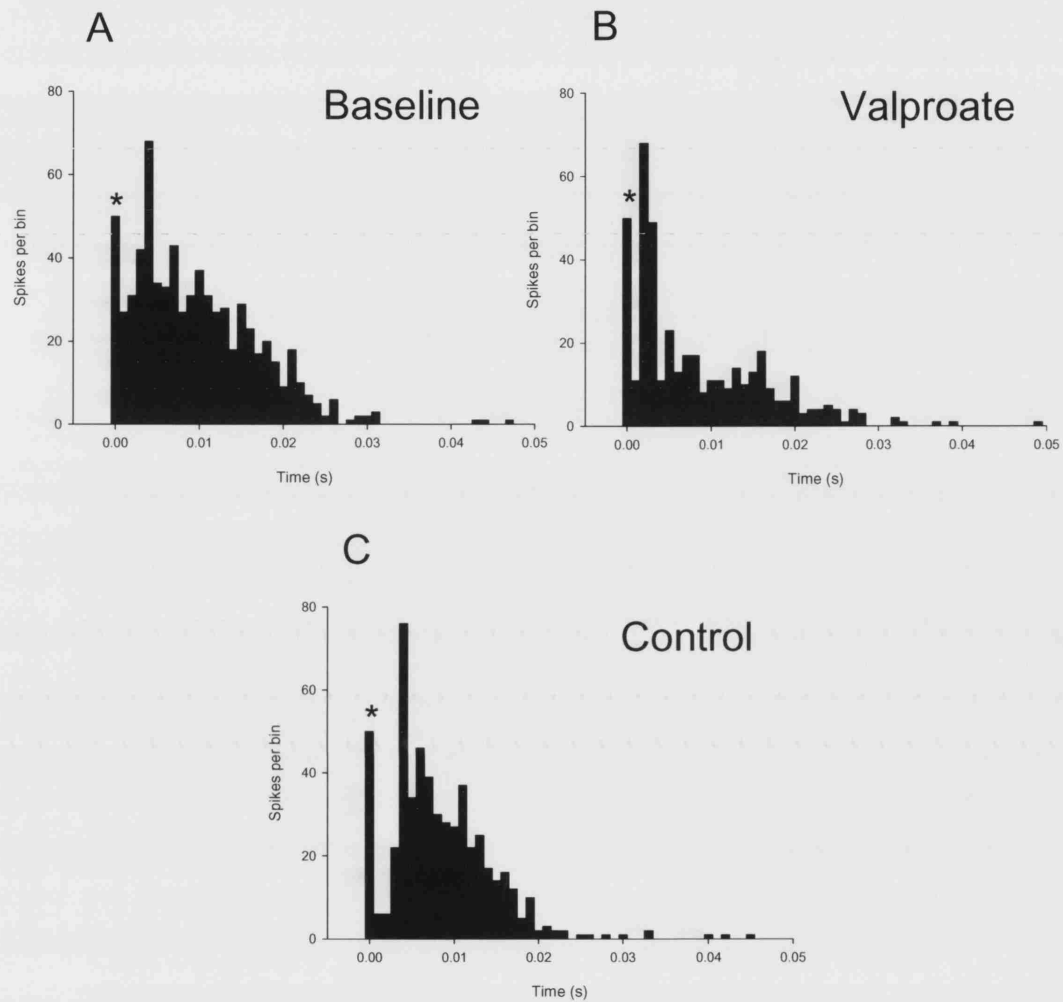


Figure 28: SSS stimulation: effect of valproate

A representative example of a post-stimulus histogram obtained from a thalamocortical neuron following electrical stimulation of the SSS: comparison of the effects of valproate and control (Cl^- ions) relative to the baseline response.

(*Stimulus artefact)

Effect of GABA on L-glutamate evoked neuronal firing

GABA

Previous studies of the ventrobasal complex have demonstrated a significant inhibitory role for GABA in somatosensory processing. Thalamocortical cells involved specifically in trigeminovascular nociceptive transmission appear to be no different. GABA reversibly suppressed the post-synaptic response to L-glutamate in all cells tested ($n = 28$, $P < 0.001$) (Table 20, Fig. 27 and 29). For individual cells this inhibition was proportionate to the magnitude of the ejecting current and by inference dose dependent ($n = 6$), though the sensitivity to GABA did vary between cells (Fig. 27 and 28). The inhibitory action of GABA on the L-glutamate response could be partially antagonised by the ejection of the selective GABA_A antagonist bicuculline ($n = 9$, $P = 0.005$). Hydroxysaclofen (GABA_B antagonist) however had no discernable effect on the inhibition produced by GABA ($n = 8$, $P = 0.8$) (Table 21, Fig. 30, 31, 36).

GABA_A and GABA_B selective agonists

We had already established that GABA exerted a significant inhibitory effect through activation of GABA_A receptors; however we confirmed this using the selective GABA_A agonist muscimol. Ejection of muscimol inhibited the response to L-glutamate in all cells tested ($n = 6$, $P = 0.001$) (Table 20, Fig. 29). This was reversible and could be antagonised with the GABA_A antagonist bicuculline ($n = 4$, $P = 0.05$), resulting in an increase in the response above the baseline (Table 21, Fig. 32). Despite an apparent lack of effect on the part of a GABA_B antagonist to even partially block the actions of GABA, we found that baclofen inhibited all cells activated by L-glutamate ($n = 11$, $P < 0.001$) (Table 20 and Fig. 29). To ensure that this was not a non-specific response we demonstrated that it could be partially antagonised by hydroxysaclofen ($n = 7$, $P = 0.01$) (Table 21, Fig. 33).

Effects of sodium valproate on L-glutamate evoked neuronal firing

Ejection of valproate was able to inhibit the L-glutamate response relative to the control ($n = 14$, $P < 0.001$) in all cells tested. There was a variable latency in the onset of its inhibitory actions, ranging (somewhat surprisingly) from an almost immediate

onset to a delay of 3 to 4 minutes. The resultant inhibition was often prolonged, lasting up to 8 to 10 minutes (Table 20, Fig. 34 and 35).

Table 20: Effects of drug ejection on L-glutamate evoked relay cell firing

i) Mean firing rates in response to L-glutamate ejection

Drug	Baseline	Control (Na ⁺ , Cl ⁻)	Drug
GABA	65 ± 7	69 ± 10	6 ± 1*
Muscimol (GABA _A agonist)	69 ± 11	68 ± 7	8 ± 4*
Baclofen (GABA _B agonist)	73 ± 9	67 ± 8	20 ± 4*
Valproate	60 ± 6	62 ± 6	23 ± 3*

Action potential firing rates (Hz) of third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result ± SEM (* $P < 0.05$).

ii) Statistical results

Drug	Drug vs. Control	Control vs. Baseline
GABA	$n = 28$, $F_{1,2,3} = 47$, $P < 0.001$	$F_{1,3} = 0.6$, $P = 0.5$
Muscimol (GABA _A agonist)	$n = 6$, $F_{1,4} = 63$, $P = 0.001$	$F_{1,4} = 0.04$, $P = 0.8$
Baclofen (GABA _B agonist)	$n = 11$, $F_{1,4} = 63$, $P < 0.001$	$F_{1,2,4} = 2$, $P = 0.2$
Valproate	$n = 14$, $F_{1,2,2} = 122$, $P < 0.001$	$F_{1,4} = 0.2$, $P = 0.7$

F and P values calculated for each treatment group comparing the effects of GABA agonists and control during co-ejection with L-glutamate. The effect of control ejection on the baseline response was also studied.

Table 21: Antagonism of selective GABA agonists by co-ejection of antagonists**i) Mean firing rates in response to L-glutamate ejection**

GABA agonist	Baseline	Agonist	Agonist and Bicuculline	Agonist and Hydroxysaclofen
GABA	66 ± 10	8 ± 3	44 ± 11*	7 ± 1
Muscimol	68 ± 4	12 ± 5	111 ± 9*	NA
Baclofen	86 ± 11	23 ± 6	NA	69 ± 18*

Action potential firing rates (Hz) of third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells in each treatment group during the test conditions were pooled to calculate the mean result ± SEM (* $P < 0.05$).

ii) Statistical results

GABA agonist	Agonist and Bicuculline	Agonist and Hydroxysaclofen
GABA	$n = 9, F_{1,9} = 15,$ $P = 0.005$	$n = 8, F_{1,4} = 0.1,$ $P = 0.8$
Muscimol	$n = 4, F_{1,1.5} = 54,$ $P = 0.005$	NA
Baclofen	NA	$n = 7, F_{1,4} = 13,$ $P = 0.01$

F and P values calculated for each treatment group comparing the effects of GABA agonists and agonists with concurrent ejection of antagonist.

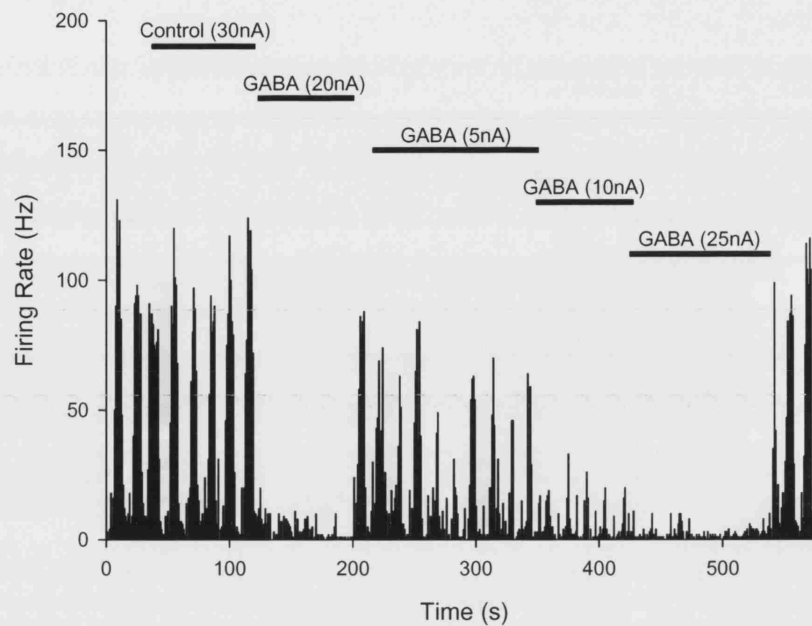


Figure 29: GABA inhibition of L-glutamate firing: dose dependency

Inhibition of the response of a third order relay cell to ejection of L-glutamate by co-ejection of GABA at different ejection currents (nA). Ejection of control (Na^+ ions) had no effect on the firing rate, but the inhibition produced by GABA increased in parallel with the magnitude of ejection current.

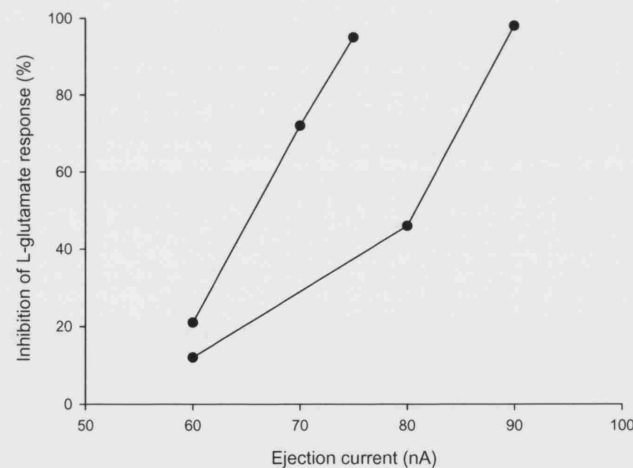


Figure 30: GABA dose: response curves

The inhibition of L-glutamate evoked neuronal firing by GABA was proportionate to its ejection current. The percentage inhibition was calculated relative to the maximum inhibition caused by ejection of GABA. Two representative examples are shown. The inhibition produced by GABA varied between cells – which may also reflect differences in anaesthetic depth.

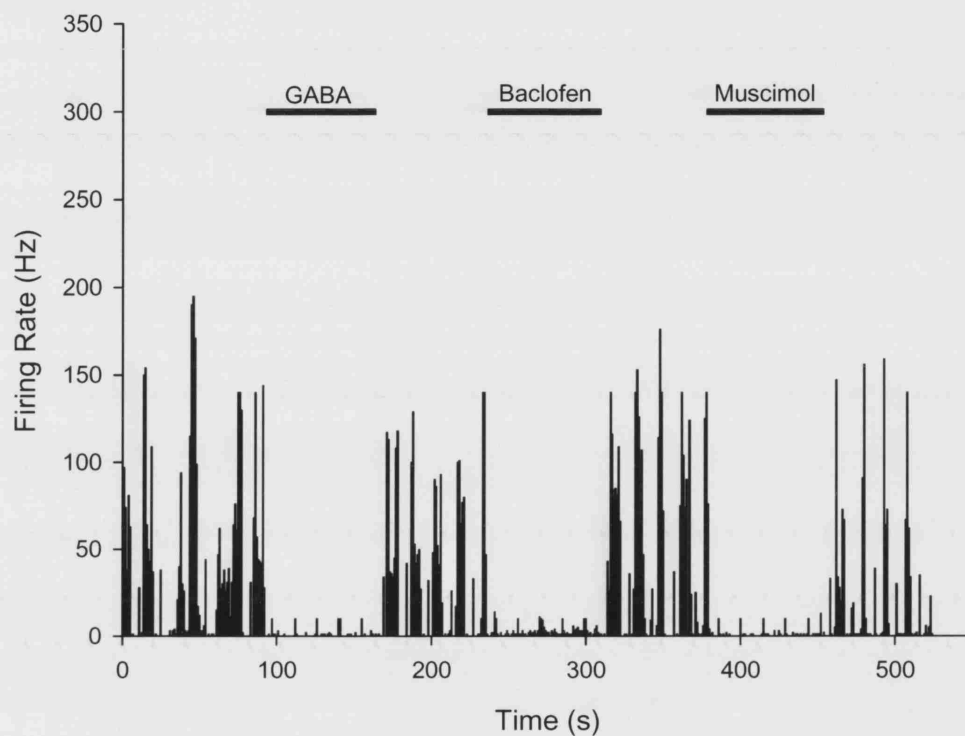


Figure 31: GABAergic (GABA_A and GABA_B) inhibition of L-glutamate firing

Sequential application of GABA, the selective GABA_B agonist baclofen and muscimol (GABA_A agonist) inhibited the response of this thalamocortical neuron to ejection of L-glutamate. Each agonist was ejected at a comparable current (+20 nA) producing a reversible inhibition. The effect of control ejection was not demonstrated in this particular tracing. This demonstrates that both GABA_A and GABA_B receptor mediated effects are present in the same cell.

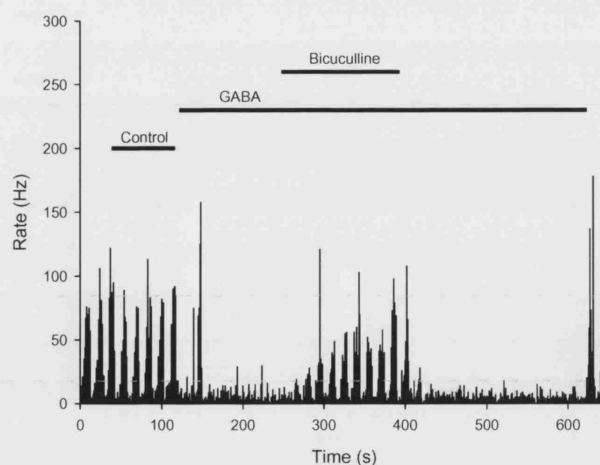


Figure 32: Bicuculline antagonises effects of GABA (L-glutamate)

Antagonism of the inhibition produced by GABA following co-ejection of the GABA_A antagonist bicuculline. Ejection of control had no effect on the baseline response to L-glutamate. The inhibition produced by GABA was reversible and could be antagonised partially by bicuculline (Control: Na⁺ ejection).

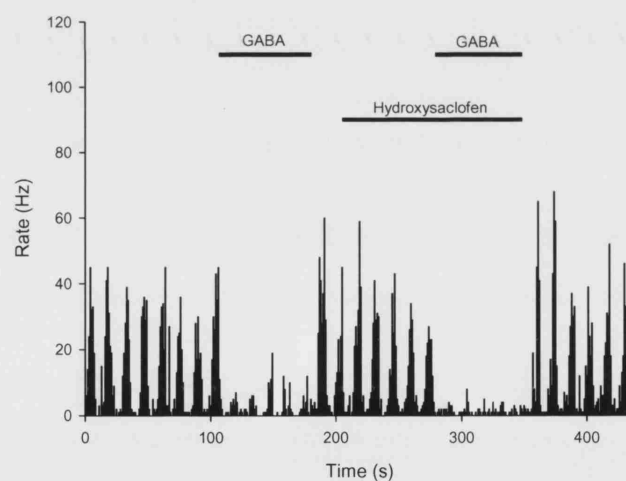


Figure 33: GABA and OH-saclofen: lack of antagonism (L-glutamate)

Ejection of OH-saclofen could not antagonise the inhibitory effect of GABA on the response to ejection of L-glutamate.

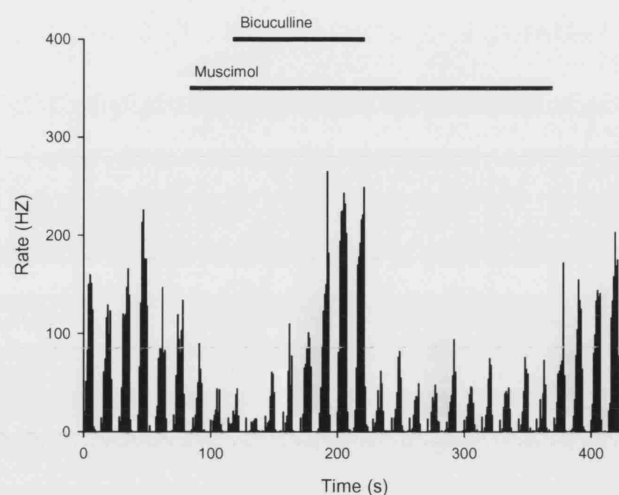


Figure 34: Selective GABA_A inhibition of L-glutamate firing

Antagonism of the inhibitory effect of the selective GABA_A agonist muscimol by bicuculline – a selective GABA_A antagonist. Note that the firing rate increases above the baseline response following ejection of bicuculline with muscimol. This was reversed when the ejection of bicuculline was stopped.

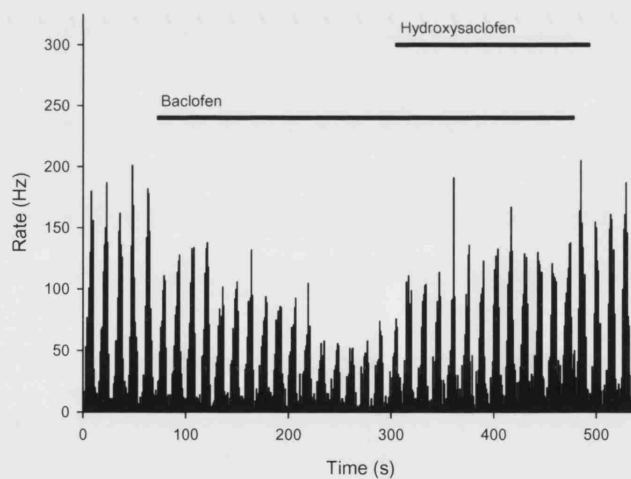


Figure 35: Selective GABA_B inhibition of L-glutamate firing

Antagonism of the inhibitory effects of baclofen (a selective GABA_B agonist) by co-ejection of hydroxysaclofen, a selective GABA_B antagonist.

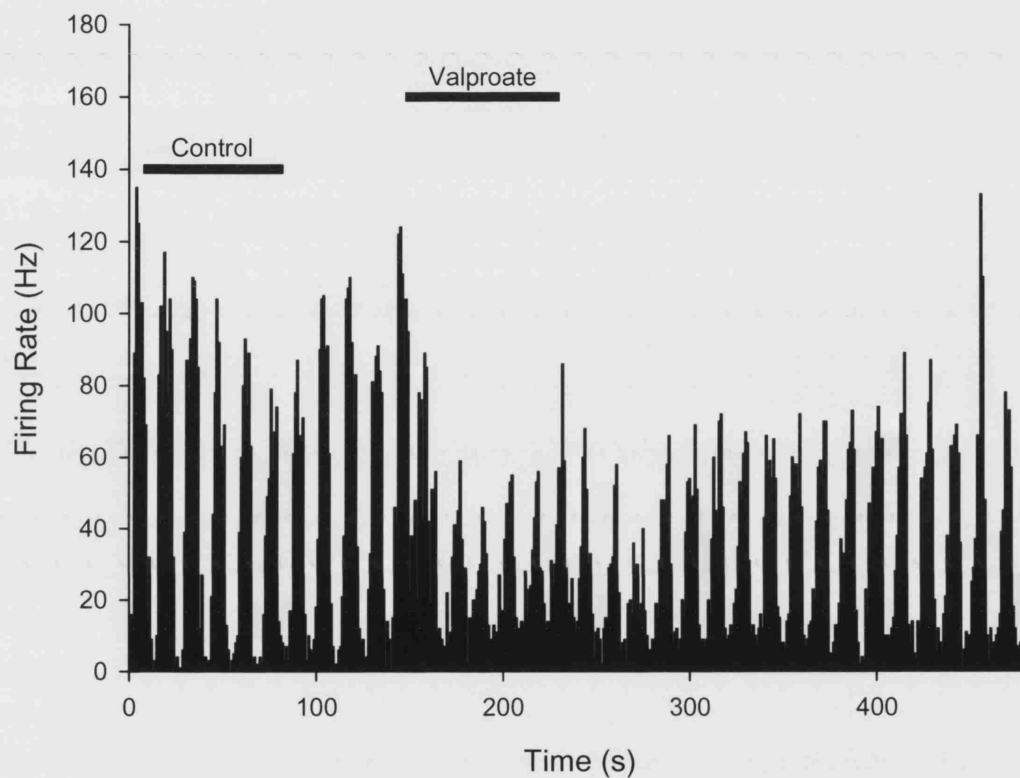


Figure 36: Inhibition of L-glutamate firing: valproate

Inhibition of neuronal firing in response to ejection of L-glutamate by co-ejection of sodium valproate. In this instance valproate ejection had an immediate effect (in other cells the onset of the inhibition had a longer latency; see discussion for further details). The firing rate of this cell did not return fully to baseline levels in the time-frame pictured (Control: Cl⁻ (Control: ejection of Cl⁻)).

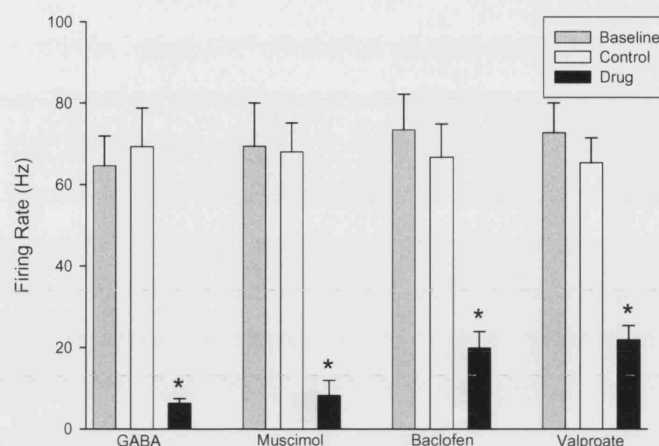


Figure 37: Inhibition of L-glutamate firing: a comparison (pooled data)

Comparison of the inhibitory actions of GABA, the selective agonists muscimol (GABA_A) and baclofen (GABA_B) and the putative GABAergic drug sodium valproate (Control: ejection of Na^+ or Cl^- ions (See Table 17)). Action potential firing rates were calculated for third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the test conditions were pooled to calculate the mean response \pm SEM. (GABA $n = 28$, Muscimol $n = 6$, Baclofen $n = 11$, Valproate $n = 14$; * $P < 0.05$).

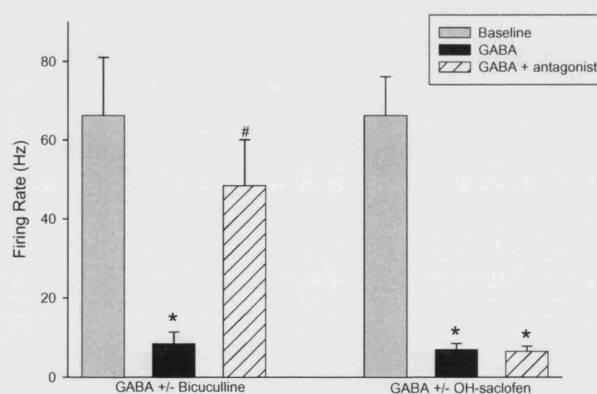


Figure 38: Selective GABA antagonists: a comparison of effects on L-glutamate firing (pooled data)

Antagonism of the inhibitory effects of GABA ejection on the response to L-glutamate. Co-ejection of bicuculline but not hydroxysaclofen (GABA_B antagonist) had an effect (GABA + Bicuculline $n = 9$, GABA + Hydroxysaclofen $n = 8$; * $P < 0.05$, * relative to baseline response, # relative to GABA response).

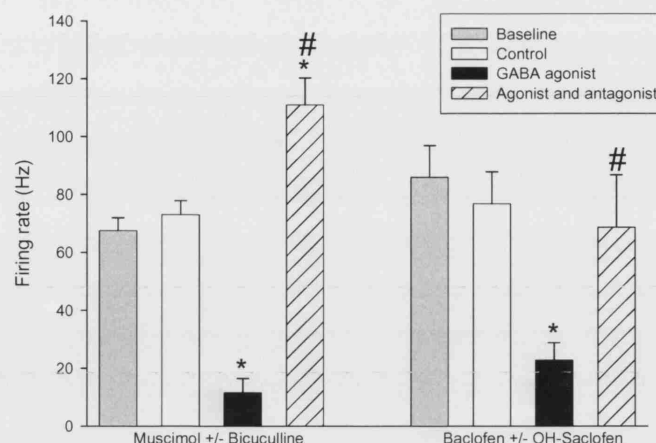


Figure 39: GABA_A and GABA_B inhibition of L-glutamate firing: a comparison

Inhibition of L-glutamate evoked firing by selective GABA agonists, and antagonism by selective antagonists (Muscimol + Bicuculline $n = 4$, Baclofen + Hydroxysaclofen $n = 7$ *# $P < 0.05$, * relative to baseline, # relative to selective GABAergic agonist).

Effects of pH

To ensure that the pH of solutions did not modulate neuronal responses, the effect of saline ejection at pH 3.5, 7.4 and 9.0 was compared using different ejection polarities - both anionic and cationic. The baseline response following L-glutamate ejection was compared to the response following Na⁺ and Cl⁻ ion ejection at pH 7.4. In neither case did ejection of Na⁺ ($P = 0.6$) or Cl⁻ ($P = 0.2$) ions have a significant effect.

The addition of 0.01M HCl, (adjusting the pH to 3.5) had no significant effect in comparison to the neutral solution. When ejected as a cation, there was no difference between the effect of Na⁺ and Na⁺/H⁺ ions ($P = 0.4$). When the solution was subjected to a current which ejected anions, acidification did not result in a significant alteration in the firing rate ($P = 0.5$) (though this is less relevant as acidic solutions are generally ejected as cations) (Table 22, Fig. 38).

A similar series of microiontophoretic experiments were performed to study the effect of addition of OH⁻ ions (in the form of 0.01M NaOH) to NaCl. When the polarity of the ejection current was adjusted to eject anions, Cl⁻ and OH⁻ ions (NaCl pH 9) had a small but non-significant effect in comparison to Cl⁻ ions (NaCl pH 7.4) ($P = 0.07$). When the same solutions were ejected as cations there was again no difference between the firing rates of thalamocortical neurons ($P = 0.5$).

Table 22: Effects of pH on the response to ejection of L-glutamate.

i) Mean firing rates following L-glutamate ejection

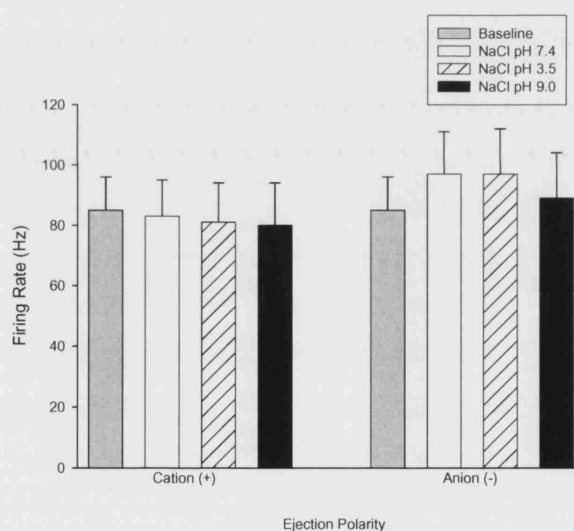
Ejection Polarity	Baseline	NaCl pH 7.4	NaCl pH 3.5	NaCl pH 9.0
Cation (+)	85 ± 11	83 ± 12	81 ± 13	80 ± 14
Anion (-)	85 ± 11	97 ± 14	97 ± 15	89 ± 15

Action potential firing rates (Hz) of third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result ± SEM (* $P < 0.05$).

ii) Statistical results

Ejection Polarity	Baseline vs. NaCl pH 7.4	NaCl pH 7.4 vs. NaCl pH 3.5	NaCl pH 7.4 vs. NaCl pH 9.0
Cation (+)	$F_{1,1.2} = 0.4, P = 0.6$	$F_{1,4} = 0.7, P = 0.4$	$F_{1,4} = 0.6, P = 0.5$
Anion (-)	$F_{1,4} = 3, P = 0.2$	$F_{1,4} = 0.002, P = 1$	$F_{1,4} = 4, P = 0.1$

F and P values calculated for each treatment group comparing the effects of NaCl at different pHs on the firing rate in response to ejection of L-glutamate.

**Figure 40: pH and ejection polarity effects: comparison with control (pooled data)**

Comparison of the effects of pH on the mean firing rate of thalamocortical neurons following ejection of L-glutamate. NaCl (0.2M) solutions of different pHs (3.5, 7.4 and 9.0) were compared. Irrespective of the ejection polarity of the solutions (as either anions (-) or cations (+)) the pH had no effect on the neuronal response.

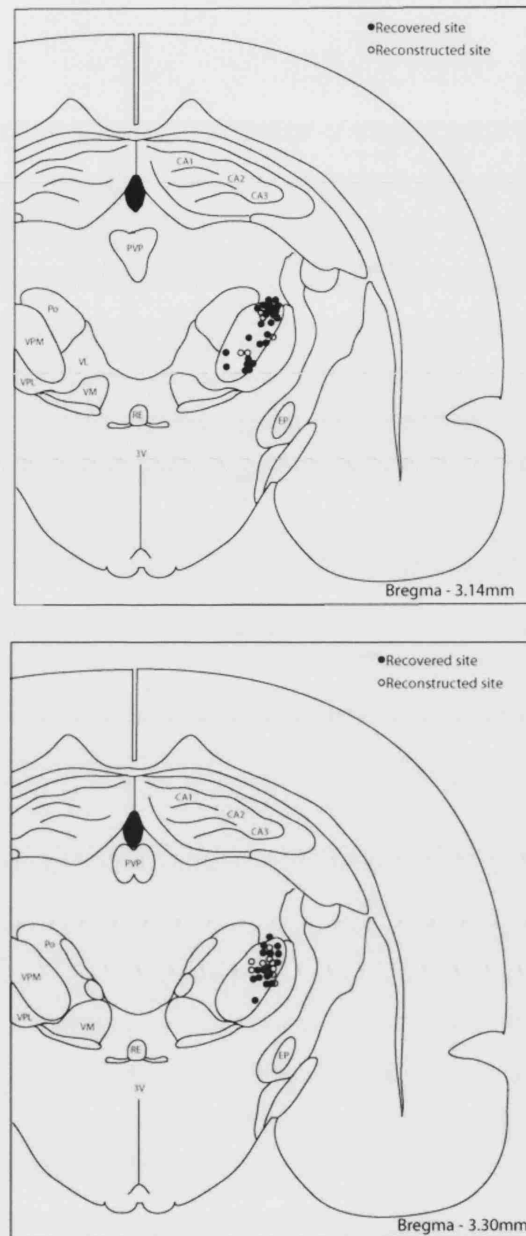


Figure 41: Histological confirmation of recording sites

Reconstruction of recording sites from lesions (filled circles) and by calculation (open circles) from microdrive readings. Adapted from (Paxinos and Watson 1986).

Abbreviations:

CA1, CA2, CA3

EP

Po

PVP

RE

VPL

VPM

VM

3V

Hippocampus CA1, CA2 and CA3

Entopeduncular nucleus

Posterior thalamic nuclear group

Paraventricular nuclear group

Reuniens nucleus

Ventroposterolateral thalamus

Ventroposteromedial thalamus

Ventromedial nuclear group

Third ventricle

Discussion

This study demonstrates for the first time that GABAergic neurotransmission in the VPM nucleus may potentially modulate trigeminovascular nociception. Furthermore it demonstrates that sodium valproate is also able to inhibit trigeminovascular nociception in the thalamus.

Anaesthetic considerations

These studies used sodium pentobarbitone to induce and maintain anaesthesia. This may be criticised as pentobarbitone possesses GABAergic properties. Barbiturate anaesthetic agents can bind to an allosteric modulation site on the GABA_A receptor. This may result in the opening of the GABA_A receptor chloride channel in the absence of an agonist. Most general anaesthetics however, with the possible exception of ketamine and xenon, affect neuronal excitability by enhancing the function of GABA_A receptors at clinically relevant concentrations (Franks et al., 1998; Franks and Lieb, 1994). In addition pentobarbitone has not demonstrated antinociceptive properties in several studies (Reyes-Vazquez et al., 1986; Vaught et al., 1985). Though pentobarbitone has undoubtedly modified the responses of VPM neurons – probably through enhancement of GABAergic neurotransmission – the effect is probably comparable to that of most commonly used anaesthetic agents. In future experiments volatile anaesthetics such as halothane or isoflurane however may be preferable to pentobarbitone as they have less of an effect on GABA receptors.

Modulation of trigeminal nociception by GABA and valproate

Microiontophoresis of GABA reversibly suppressed neuronal firing in response to nociceptive intracranial stimulation. GABA also reversibly inhibited neuronal activity in response to L-glutamate. GABA's action on the L-glutamate evoked response had a very sudden onset of action. Likewise the inhibition was reversed quite abruptly upon cessation of ejection - presumably due to either rapid diffusion of GABA from its site of action or uptake into nerve terminals. In a number of cells GABA inhibited the L-glutamate response in a "dose-dependant" manner. The quantity of drug ejected by microiontophoresis is proportionate to the total charge flux during the ejection period. Given that these particular experiments examined the same neurons at variable ejection currents, other variables – such as transport number and diffusion constant of

GABA - should be constant. It is therefore possible to equate the ejection current to the quantity of GABA that the cell is exposed to.

As was expected GABA_A receptor activation played a major role in GABA's antinociceptive properties. The inhibitory actions of GABA could be antagonised by co-ejection of the GABA_A antagonist bicuculline. Ejection of bicuculline often resulted in an increase in a neuron's spontaneous firing rate. The firing rate of neurons following L-glutamate ejection often increased above baseline levels during co-ejection of bicuculline. Ejection of hydroxysaclofen however was not able to antagonise the inhibitory action of GABA. Superficially this would suggest that GABA inhibits neuronal firing in response to ejection of L-glutamate predominately through activation of GABA_A receptors, though as will be discussed later this may be an overly simplistic interpretation of the experimental data.

Muscimol, a selective GABA_A agonist also reversibly inhibited the response to L-glutamate in every cell tested. Like GABA, the inhibition produced by muscimol had a rapid time course - this is not surprising given the ionotropic nature of GABA_A receptors. The inhibition caused by muscimol was antagonised in every cell by co-ejection of bicuculline. It was often observed that bicuculline ejection increased both spontaneous and L-glutamate evoked neuronal firing. This would suggest that GABA_A receptors are tonically activated in this population of thalamocortical neurons.

A possible role for the GABA_B agonist baclofen in the thalamus was also identified. Baclofen inhibited L-glutamate evoked firing in every neuron tested. Baclofen did not generally have quite as rapid a time-course of action as muscimol and GABA. The response generally progressed at a slower pace which may reflect a delay due to the limitations of diffusion from the barrel tip. In a small number of studies ejection of both muscimol and baclofen inhibited the response to L-glutamate in the same cells. It therefore appears likely that populations of both GABA_A and GABA_B receptors are found on the same thalamocortical neurons, mediating the inhibitory actions of GABA.

It was not possible to study the effects of microiontophoretically ejected baclofen on the response to SSS stimulation as "barrel blocking" repeatedly prevented it. When we studied the effect of a drug on neuronal firing secondary to SSS stimulation the drug in question had to be ejected for prolonged periods – generally for between 3 to 5 minutes – before an effect was seen. This was much longer than that required when examining the effect on L-glutamate evoked firing. Unfortunately the longer that a

drug is ejected the more likely it is that the barrel will “block”. All studies examining the effects on SSS stimulus evoked firing were complicated by this technical limitation - though some drugs were more prone to it than others. Despite this significant limitation these experiments suggest an anti-nociceptive role for GABA_B agonists in the thalamus which would be in keeping with other studies (Ipponi et al., 1999; Olausson et al., 1994). It may seem difficult however to reconcile a role for GABA_B receptor activation in this model given the inability of hydroxysaclofen to reverse the effects of GABA. Hydroxysaclofen may have been an insufficiently potent antagonist to block the actions of GABA. Alternatively, given that this technique is ultimately dependent on diffusion, adequate quantities of hydroxysaclofen may not have reached a sufficient number of synapses. The mechanisms of action of the two receptors may also partially explain this discrepancy. Though activation of GABA_A and GABA_B receptors results in neuronal hyperpolarisation, they have very different effects on membrane conductance and ionic flux. Activation of GABA_A receptors results in a much larger increase in membrane conductance than following GABA_B receptor activation. This “shunts” the membrane potential towards the reversal potential for chloride ions. It may be that this would obscure any effect brought about by antagonism of GABA_B receptors. Baclofen also persists in the extracellular space as it is not subject to presynaptic uptake unlike GABA. One could speculate that persistent agonism of GABA_B receptors may produce an unphysiological response, leading us to over estimate the functional effects of GABA_B receptor activation. We can also not exclude the possibility that baclofen may be acting presynaptically on nerve terminals which are also being activated by ejection of L-glutamate. As ejection of L-glutamate may trigger the release of excitatory neurotransmitters from presynaptic terminals it is conceivable that GABA_B receptors may not have a significant function on thalamocortical neurons; rather what we are seeing is a pre-synaptic action on nerve terminals which are being stimulated in an unphysiological manner. These issues could however be partially resolved if we demonstrated that neuronal firing secondary to SSS stimulation could be blocked by ejection of a GABA_B agonist.

Sodium valproate was able (like GABA) to inhibit the response of third order neurons to SSS stimulation and L-glutamate. Like GABA agonists, valproate also has antinociceptive properties (Guieu et al., 1993; Mesdjian et al., 1983). It is possible that valproate inhibits trigeminovascular nociceptive transmission in the VPM by augmenting endogenous GABAergic inhibition. This may explain why the onset of

inhibition was occasionally delayed - and the prolonged length of the inhibition. This however remains a matter of speculation as the pharmacological action of valproate (be it GABAergic or by other mechanisms) was not specifically studied. This unfortunate limitation means that other non-GABAergic modes of action of valproate cannot be excluded as a cause for the observed inhibition. Further studies will be needed to confirm the GABAergic nature of this inhibition. One method may involve concurrent ejection of selective GABA_A and GABA_B antagonists. If the inhibitory effect of valproate ejection could be blocked by bicuculline or hydroxysaclofen this would support a GABAergic mode of action.

A further limitation of this study is that we can only examine the effects of valproate and baclofen on neuronal firing after short periods of ejection. Valproate and baclofen are generally used as prophylactic agents. Sodium valproate has been used effectively as an abortive migraine treatment, but no equivalent effect has been reported for baclofen. There is no way currently of modelling chronic exposure using this technique. Chronic administration of GABAergic drugs may have complex effects on GABA receptor density and kinetics. Studies will have to be performed to address this issue and examine if trigeminovascular nociceptive neurotransmission is significantly altered as a result.

The pH of most drug solutions must be adjusted to facilitate drug ejection. Any effect of H⁺ or OH⁻ ejection would be a confounding variable in all microiontophoretic studies. Importantly ejection of Na⁺/H⁺ ((+) ejection polarity) or Cl⁻/OH⁻ ((-) ejection polarity) ions does not have a significant effect in comparison to Na⁺ or Cl⁻ ions (at neutral pH) on the baseline response following L-glutamate ejection. Though Cl⁻ ions had a mild facilitatory effect on neuronal firing this was not statistically significant. The addition of small quantities of HCl and NaOH to the drug solutions in these experiments therefore had no effect on the responses of thalamocortical neurons following L-glutamate ejection.

In conclusion we propose that the VPM nucleus is likely involved in the transmission of painful sensory information to the cortex in migraine. GABA modulates this flow of sensory information and it appears that both GABA_A and GABA_B receptors may be involved in this process. Sodium valproate also inhibits trigeminal nociceptive transmission in the VPM.

Study 2: Investigation of the modulatory actions of naratriptan and ergometrine maleate on trigeminovascular nociception by thalamocortical neurons of the rat VPM nucleus.

Introduction

One of the most significant advances in our understanding and treatment of migraine has been the development of the triptans (Davidoff, 2002). The triptans are very effective abortive treatments for migraine. Primarily designed as agonists of $5\text{-HT}_{1B/1D}$ receptors, sumatriptan is the prototypical drug in this class. Second generation triptans such as naratriptan, zolmitriptan and rizatriptan are now widely used in clinical practice. Second generation triptans are more lipophilic, and hence brain penetrant, than sumatriptan and differ in their pharmacokinetic properties (Davidoff, 2002).

Opinions differ regarding the principal site of action of the triptans. They modulate the activity of both first and second order trigeminovascular nociceptive neurons in addition to their well described vasoconstricting actions on blood vessels (Goadsby et al., 2002b; Storer and Goadsby, 1997). To date however no study has investigated possible actions of these drugs on higher centres of trigeminal nociceptive processing. 5-HT_1 receptors are typical G-protein coupled receptors which negatively regulate adenylate cyclase activity through G_i/G_o proteins. They modulate neuronal excitability through actions on a diversity of enzymatic pathways and ion channels. They are located both pre- and post-synaptically acting as auto- and heteroreceptors, regulating the activity of serotonergic and non-serotonergic neurons (Barnes and Sharp, 1999).

This study investigated whether ejection of naratriptan and ergometrine maleate could inhibit thalamocortical activity following superior sagittal sinus stimulation and L-glutamate evoked third order neuronal firing. It also examined the pharmacological mechanisms by which naratriptan exerted this effect through actions on 5-HT_1 receptors (Table 23). As triptans have agonist activity at most 5-HT_1 receptor subtypes, antagonists of 5-HT_{1A} and $5\text{-HT}_{1B/1D}$ receptors were co-ejected with naratriptan. If the actions of naratriptan could be antagonised, it would strongly suggest that activation of those particular receptors contributed to the action of naratriptan.

Table 23: Summary of drugs used in Study 2

Drug	Pharmacological Action	Ejection Polarity	Control
Naratriptan	5-HT ₁ agonist	(+)	Na ⁺ , H ⁺ ions
Ergometrine	5-HT, noradrenergic and dopaminergic agonist	(+)	Na ⁺ , H ⁺ ions
8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT)	5HT _{1A} agonist	(+)	Na ⁺ , H ⁺ ions
GR 127935	5-HT _{1B/1D} antagonist	(+)	Na ⁺ , H ⁺ ions
(S)-WAY 100135	5-HT _{1A} antagonist	(+)	Na ⁺ , H ⁺ ions

(+): cation

Results

A total of 48 cells were studied (Table 24). Cells responded with an increased probability of firing to electrical superior sagittal sinus stimulation with a mean response probability of $73 \pm 3\%$ (mean \pm S.E.M). Recording sites are shown in Fig. 54.

Table 24: Receptive field characteristics of the neurons in Study 2

Receptive Field Characteristics	Number of cells
Low threshold mechanosensitive (LTM)	11
Nociceptive specific (NS)	1
Wide dynamic range (WDR)	7
Vibrissal (no. of vibrissae: mean \pm SEM)	29 (2.4 ± 0.3)

Effects of Naratriptan

Microiontophoretic ejection of naratriptan reversibly reduced the response probability of third order neurons following SSS stimulation in comparison to the control ($n = 8$, $t_7 = 5.5$, $P = 0.001$; Table 25a, Fig. 40). Ejection of control had no significant effect on the baseline response ($P = 0.1$). Intravenous naratriptan ($n = 8$) also inhibited the response to SSS stimulation ($t_7 = 13.3$, $P < 0.001$) relative to the baseline (Table 25b, Fig. 41 and 43). The injection of naratriptan did not result in a significant change in

the subjects' blood pressure (BP: 116 ± 12 mmHg, 115 ± 11 mmHg before and 1 minute after injection (mean \pm SEM)). Peak inhibition was observed between 5-10 minutes after administration (Fig. 44). Injection of 0.9% NaCl (1ml/kg) ($n = 5$) i.v. had no effect on the response to SSS stimulation relative to the baseline response ($n = 5$, $t_4 = 0.7$, $P = 0.5$; Fig. 41).

Table 25: Effects of serotonergic agonists on the response to SSS stimulation

a) Microiontophoretic delivery

i) Mean response probabilities following SSS stimulation

Serotonergic agonist	Baseline	Control (Na⁺, H⁺ ions)	Agonist
Naratriptan	$58 \pm 8\%$	$65 \pm 10\%$	$40 \pm 6\%^*$
8-OH-DPAT	$80 \pm 8\%$	$83 \pm 9\%$	$52 \pm 9\%^*$

Response probabilities of thalamocortical neurons following electrical stimulation of the SSS. When a neuron was located a series of 50 stimuli were delivered to the SSS and the response probability (as a percentage) calculated. Results under the three test conditions were pooled and are presented as the mean \pm SEM (* P was calculated < 0.05).

ii) Statistical results

Serotonergic agonist	Agonist v. baseline	Agonist v. control	Baseline v. control
Naratriptan	$t_7 = 4$, $P = 0.005$	$t_7 = 5.5$, $P = 0.001$	$t_7 = 1.7$, $P = 0.1$
8-OH-DPAT	$t_7 = 6.8$, $P < 0.001$	$t_7 = 6.3$, $P < 0.001$	$t_7 = 0.5$, $P = 0.6$

Paired sample t -test analysis of the effects of serotonergic agonists on the response probabilities of thalamocortical neurons following electrical stimulation of the SSS.

b) Intravenous delivery

Mean response probabilities in response to SSS stimulation

Intravenous agent	Baseline	Drug	Baseline v. drug
Naratriptan	$91 \pm 3\%$	$38 \pm 5\%^*$	$t_7 = 13.3$, $P < 0.001$
NaCl	$96 \pm 2\%$	$94 \pm 3\%$	$t_4 = 0.7$, $P = 0.5$

Results were pooled and are presented as the mean \pm SEM.
(* $P < 0.05$)

Naratriptan also reversibly suppressed the response to L-glutamate following microiontophoretic ejection in all cells tested ($n = 9$, $P < 0.001$). The inhibition often only reached its maximum level following a latency of greater than 60s – it was also relatively prolonged, lasting up to 5 to 10 minutes (Table 26, Fig. 45 and 46).

The inhibitory action of naratriptan on the L-glutamate response could be partially antagonised by the simultaneous ejection of the potent 5-HT_{1A} antagonist (S)-WAY 100135 ($n = 7$, $P < 0.001$). GR 127935 (5-HT_{1B/1D} antagonist) was also able to partially block the inhibitory action of naratriptan ($n = 6$, $P = 0.01$) when the two drugs were co-ejected. Microiontophoresis of neither (S)-WAY 100135 nor GR 127935 had a significant effect on the L-glutamate response when ejected individually ($P = 0.2$; $P = 0.6$). This makes it unlikely that antagonists of 5-HT_{1A} and 5-HT_{1B/1D} receptors have stimulatory effects on L-glutamate evoked firing. It is therefore reasonable to assume that the partial reversal of the inhibition produced by naratriptan represents antagonism at these receptors (Table 27, Fig. 47 to 50).

Serotonergic 5-HT_{1A} receptor mediated effects

As the inhibitory effects of naratriptan could be antagonised by co-ejection of (S)-WAY 100135, the modulatory functions of 5-HT_{1A} receptor activation were further studied. The selective 5-HT_{1A} agonist (R)-(+)-8(OH)-DPAT, like naratriptan, reversibly reduced the response probability of thalamocortical neurons to SSS stimulation ($n = 8$, $t_7 = 6.3$, $P < 0.001$) following microiontophoretic ejection. Ejection of control again had no effect ($P = 0.6$) (Table 25a, Fig. 42 and 43). The effects of intravenous administration of (R)-(+)-8(OH)-DPAT were not studied.

(OH)-DPAT also inhibited the cellular response to L-glutamate in all cells tested ($n = 9$, $P < 0.001$). Ejection of control had no effect in comparison to the baseline ($P = 0.3$). The (R)-(+)-8 OH-DPAT inhibition of the L-glutamate response was reversible and often had a shorter time course compared to naratriptan - with little latency between the onset and termination of its inhibitory effect relative to the flow of ejecting current (Table 26, Fig. 51 and 53).

Effects of ergometrine maleate

Unfortunately we could not study the action of ergometrine on the response to SSS stimulation as we encountered the problem of barrel “blocking” whenever it was attempted. The modulatory effects of ergometrine on the response to L-glutamate ejection could therefore only be studied. Ergometrine inhibited the response to L-glutamate ($n = 6$, $P = 0.001$) – the inhibition developing with a variable latency often exceeding 1 to 2 minutes (Table 26, Fig. 52 and 53).

Table 26: Effects of serotonergic agonists on the L-glutamate evoked response.

i) Mean firing rates in response to L-glutamate ejection

Drug	Baseline	Control (Na ⁺ , H ⁺ ions)	Agonist
Ergometrine maleate	95 ± 12	89 ± 15	22 ± 6*
8-OH-DPAT	103 ± 12	98 ± 12	22 ± 7*
Naratriptan	62 ± 9	55 ± 6	17 ± 2*

Action potential firing rates (Hz) of third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result ± SEM (* P was calculated < 0.05).

ii) Statistical results

Drug	Baseline v. Control	Control v. Agonist
Ergometrine Maleate	$n = 6$, $F_{1,4} = 0.2$, $P = 0.7$	$n = 6$, $F_{1,4} = 41$, $P = 0.001$
8-OH-DPAT	$n = 9$, $F_{1,4} = 1$, $P = 0.3$	$n = 9$, $F_{1,4} = 64$, $P < 0.001$
Naratriptan	$n = 9$, $F_{1,1.9} = 2$, $P = 0.2$	$n = 9$, $F_{1,4} = 35$, $P < 0.001$

F and P values calculated for each treatment group comparing the effects of serotonergic agonists and control during co-ejection with L-glutamate. The effect of control ejection on the baseline response was also studied.

Table 27: Serotonergic receptor antagonists: effects on naratriptan (L-glutamate).**i) Mean firing rates in response to L-glutamate ejection**

Serotonergic antagonist	Baseline	Antagonist	Naratriptan	Naratriptan + antagonist
GR 127935	101 ± 14	109 ± 24	21 ± 3*	50 ± 8* #
(S)-WAY100135	82 ± 9	86 ± 7	22 ± 2*	52 ± 3* #

Action potential firing rates (Hz) of third order neurons following ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells in each treatment group during the test conditions were pooled to calculate the mean result ± SEM

(* # $P < 0.05$; * denotes significance relative to baseline values, # denotes significance relative to naratriptan values)

ii) Statistical results

Serotonergic antagonist	Baseline v. antagonist	Baseline v. naratriptan	Baseline v. naratriptan + antagonist	Naratriptan + antagonist v. naratriptan
GR 127935	$n = 5$, $F_{1,12} = 0.3$, $P = 0.6$	$n = 6$, $F_{1,13} = 39$, $P = 0.002$	$n = 6$, $F_{1,12} = 12$, $P = 0.02$	$n = 6$, $F_{1,4} = 15$, $P = 0.01$
(S)-WAY100135	$n = 7$, $F_{1,4} = 3$, $P = 0.2$	$n = 7$, $F_{1,4} = 65$, $P < 0.001$	$n = 7$, $F_{1,4} = 15$, $P = 0.008$	$n = 7$, $F_{1,19} = 193$, $P < 0.001$

F and P values calculated for each treatment group comparing the effects of naratriptan ± selective 5HT₁ antagonist during co-ejection with L-glutamate.

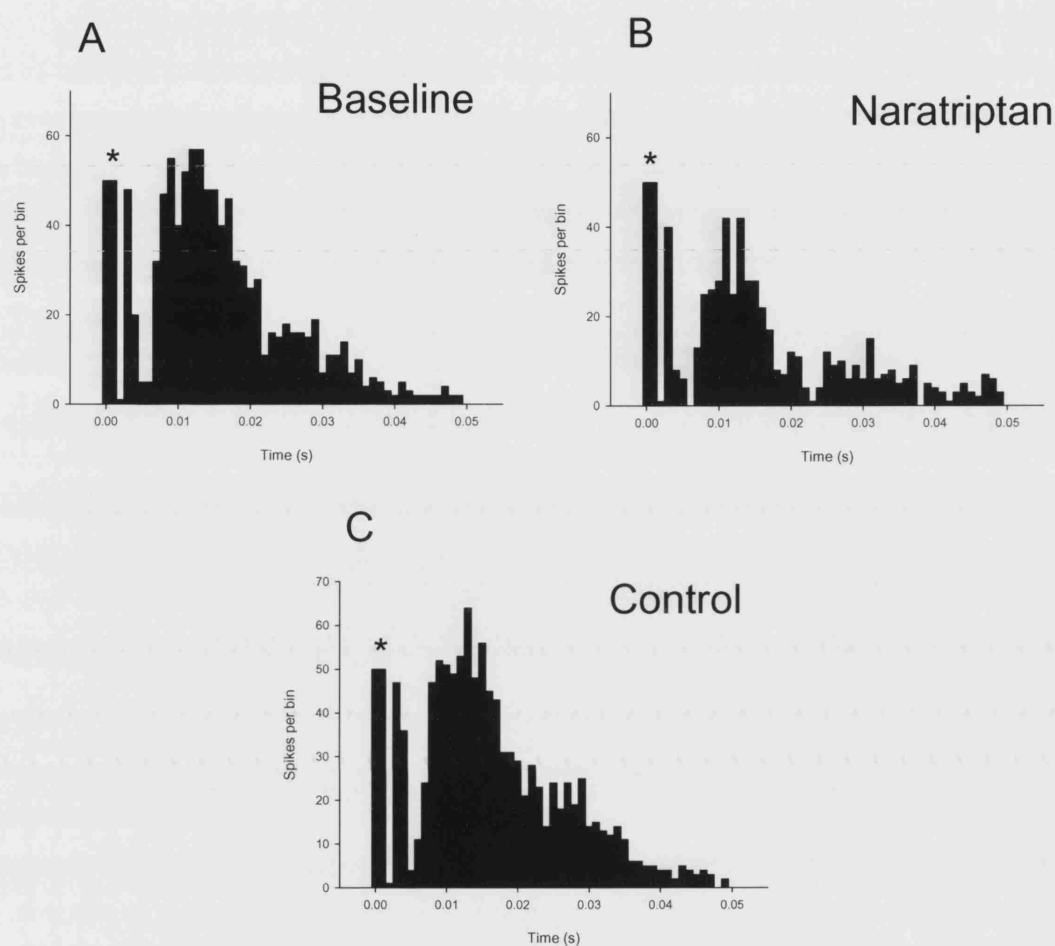


Figure 42: SSS stimulation: effects of naratriptan (microiontophoretic ejection)

A representative example of the post-stimulus histograms obtained from a thalamocortical neuron following electrical stimulation of the SSS. The inhibitory effect of naratriptan ejection in comparison to the control ejection of Na^+ and H^+ ions is clearly demonstrated.

(*stimulus artefact)

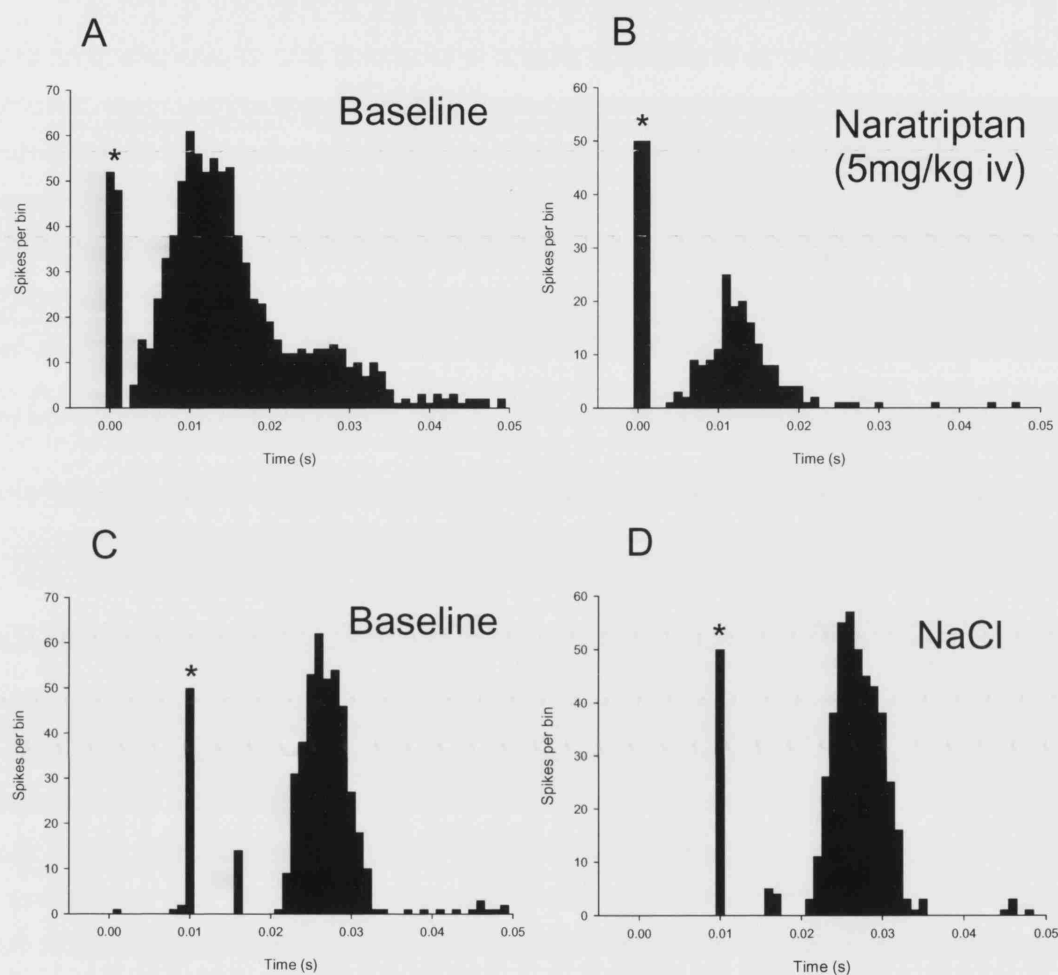


Figure 43: SSS stimulation: effects of naratriptan (intravenous)

Comparison of the effects of intravenous naratriptan (5mg/kg) and 0.9% NaCl on the response of thalamocortical neurons to electrical stimulation of the SSS. Both injections were given in a volume of 1ml/kg. Though B and D represent responses in two separate experiments, it is apparent that while naratriptan did have a marked inhibitory effect, sodium chloride did not.

(* stimulus artefact)

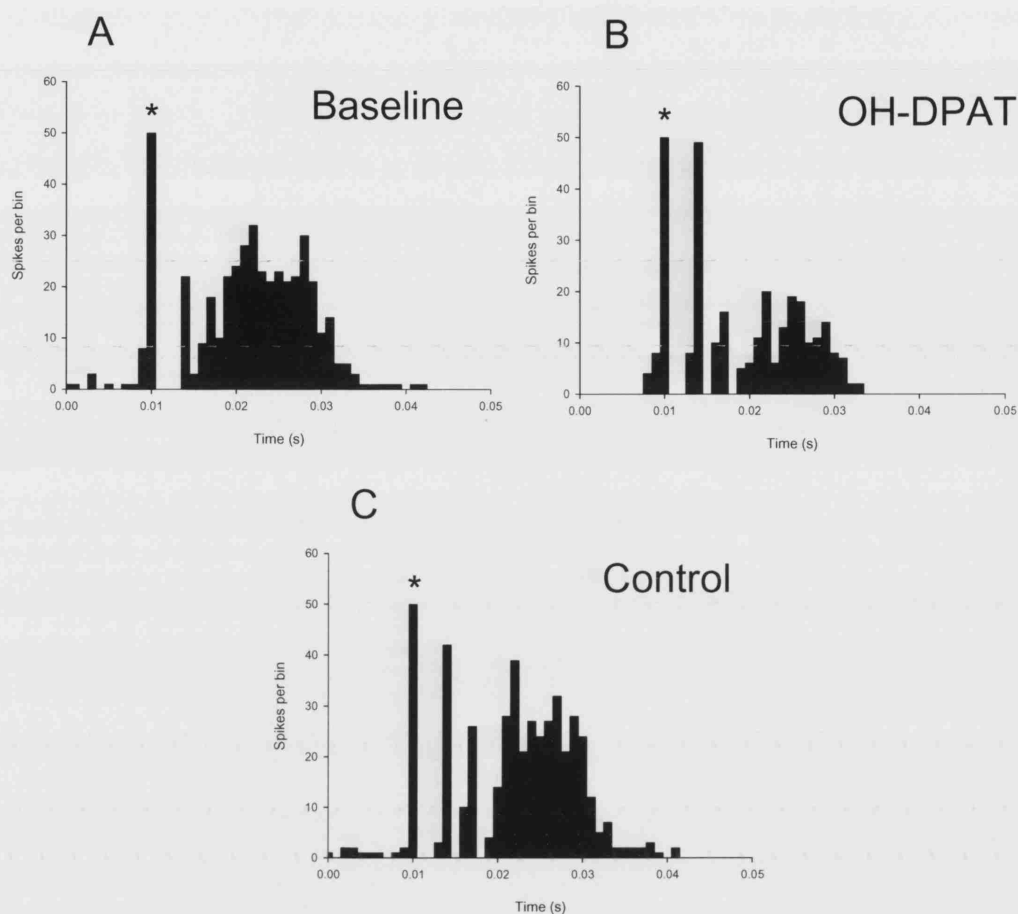


Figure 44: SSS stimulation: effects of OH-DPAT

The 5-HT_{1A} receptor agonist OH-DPAT also inhibited the response following electrical stimulation of the SSS after microiontophoretic ejection. OH-DPAT and control ejection had no effect however on the first peak (latency \approx 5-7ms) which may represent activation of corticothalamic afferents (Control: Na⁺ /H⁺ ion ejection). (*stimulus artefact)

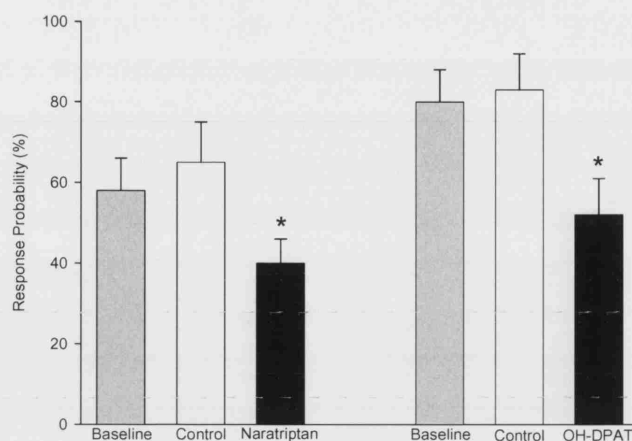


Figure 45: SSS stimulation: comparison of naratriptan and OH-DPAT

Effects of microiontophoretically delivered serotonergic agonists on the response probability (%) of thalamocortical neurons to electrical stimulation of the SSS. The mean response \pm SEM (pooled data) was calculated for each of the microiontophoretic treatment groups under the 3 conditions ($n = 8$, $*P < 0.05$).

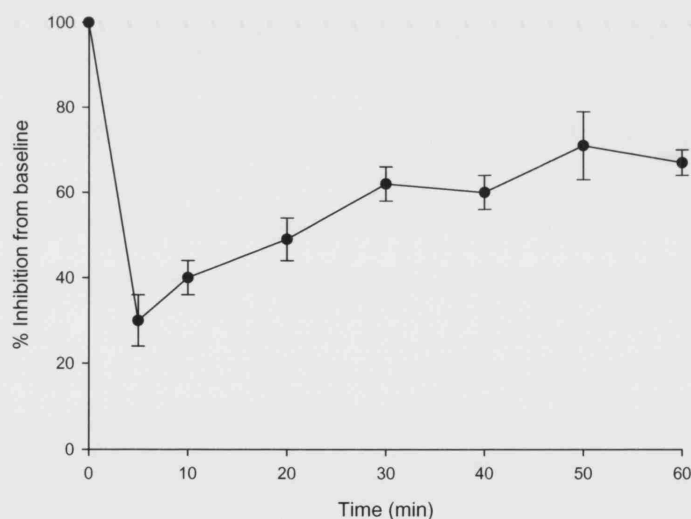


Figure 46: SSS stimulation: temporal profile of naratriptan inhibition

Temporal profile of the inhibition produced following intravenous naratriptan (5mg/kg) on the response of third order neurons following electrical stimulation of the SSS. The percentage inhibition from the baseline was calculated relative to the average baseline response probability after 50 electrical stimuli (taken to be 100%). As can be seen the maximal inhibition occurred between 5 and 10 minutes after administration of naratriptan. Mean \pm SEM values are shown.

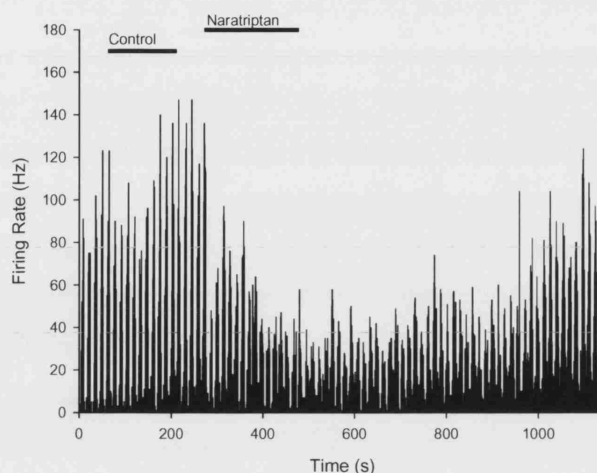


Figure 47: Inhibition of L-glutamate firing: naratriptan

Inhibition of the response of a thalamocortical neuron to ejection of L-glutamate pulses by naratriptan. Ejection of Na^+ and H^+ ions as a control had no effect on the response relative to the baseline firing rates. Naratriptan reversibly inhibited the neuronal firing rate though this did not occur immediately and substantially outlasted the ejection period.

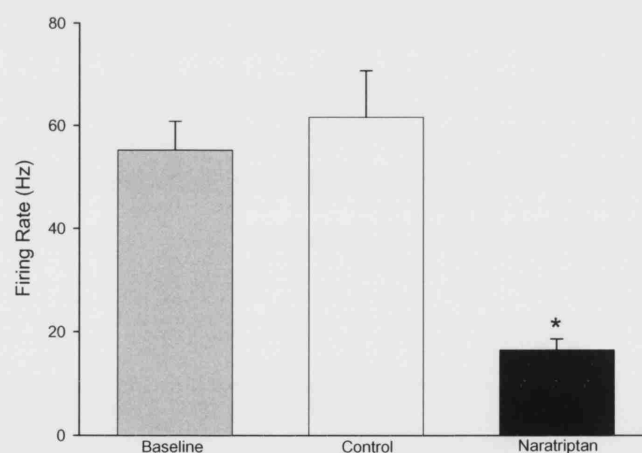


Figure 48: Naratriptan inhibition of L-glutamate firing (pooled data)

Action potential firing by third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the test conditions were pooled to calculate the mean response \pm SEM. This demonstrates the significant inhibitory effects brought about by co-ejection of naratriptan. ($n = 9$, * $P < 0.05$)

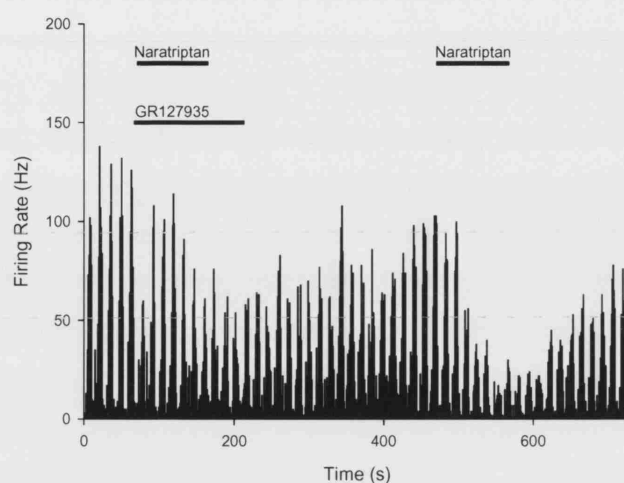


Figure 49: Naratriptan: 5-HT_{1B/1D} effects

The inhibitory effects of naratriptan on L-glutamate evoked neuronal firing could be partially blocked by the simultaneous ejection of the selective 5-HT_{1B/1D} antagonist GR 127935. This indicates that the inhibitory actions caused by ejection of naratriptan are due in part to agonism at these receptors.

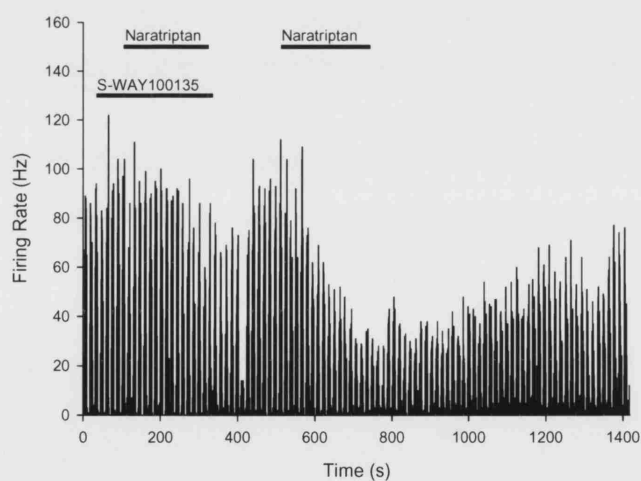


Figure 50: Naratriptan: 5-HT_{1A} effects

The selective 5-HT_{1A} antagonist S-WAY 100135 also partially antagonised the inhibitory actions of naratriptan – demonstrating that agonism of 5-HT_{1A} receptors is a component of the inhibitory actions of naratriptan.

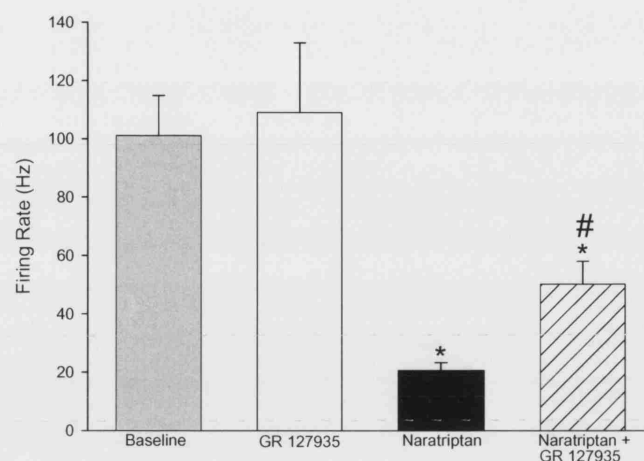


Figure 51: Naratriptan: 5-HT_{1B/1D} effects (pooled data)

Partial antagonism of the inhibitory actions of naratriptan by the 5-HT_{1B/1D} antagonist GR 127935. Though the mean response of the neurons following ejection of naratriptan and GR 127935 was still less than the baseline firing rate following a 5s pulse of L-glutamate, it was significantly higher than the response following ejection of naratriptan by itself (mean \pm SEM values are shown).

($n = 5$, * $P < 0.05$; * relative to baseline, # relative to naratriptan)

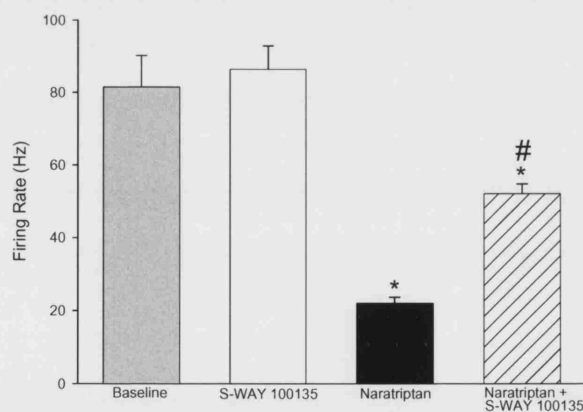


Figure 52: Naratriptan: 5-HT_{1A} effects (pooled data)

Partial antagonism of naratriptan's inhibitory effects by co-ejection of (S)-WAY 100135. As with GR 127935, firing rates were significantly decreased by naratriptan - even with co-ejection of (S)-WAY 100135. The 5-HT_{1A} antagonist did however significantly antagonise the inhibition brought about by the ejection of naratriptan (mean \pm SEM values are shown).

($n = 7$, * $P < 0.05$; * relative to baseline, # relative to naratriptan)

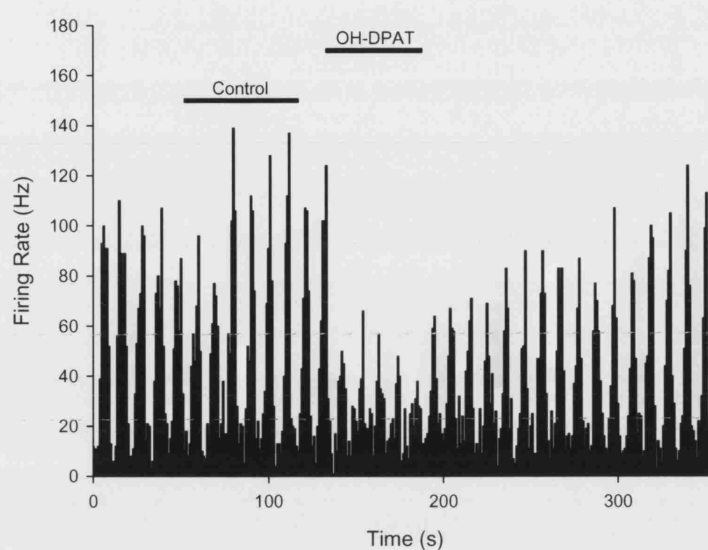


Figure 53: L-glutamate firing: inhibition by the 5-HT_{1A} agonist OH-DPAT

Inhibition of L-glutamate evoked neuronal firing by ejection of the selective 5-HT_{1A} agonist 8-(OH)-DPAT.

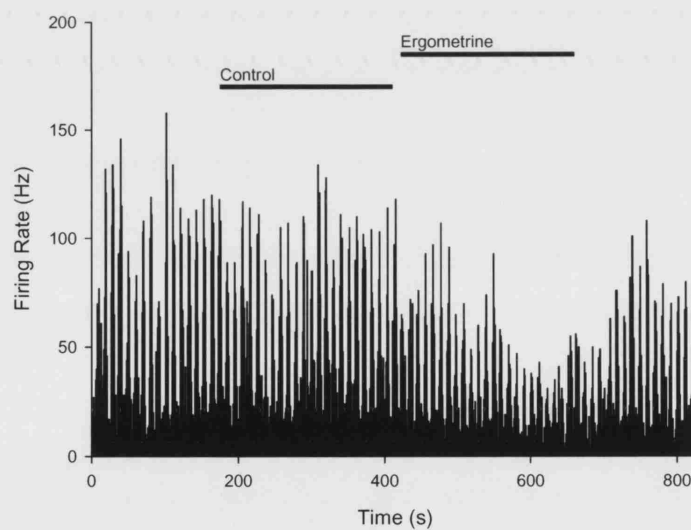


Figure 54: L-glutamate firing: inhibition by ergometrine maleate

Inhibition of L-glutamate evoked firing resulting from the co-ejection of the ergot derivative ergometrine maleate.

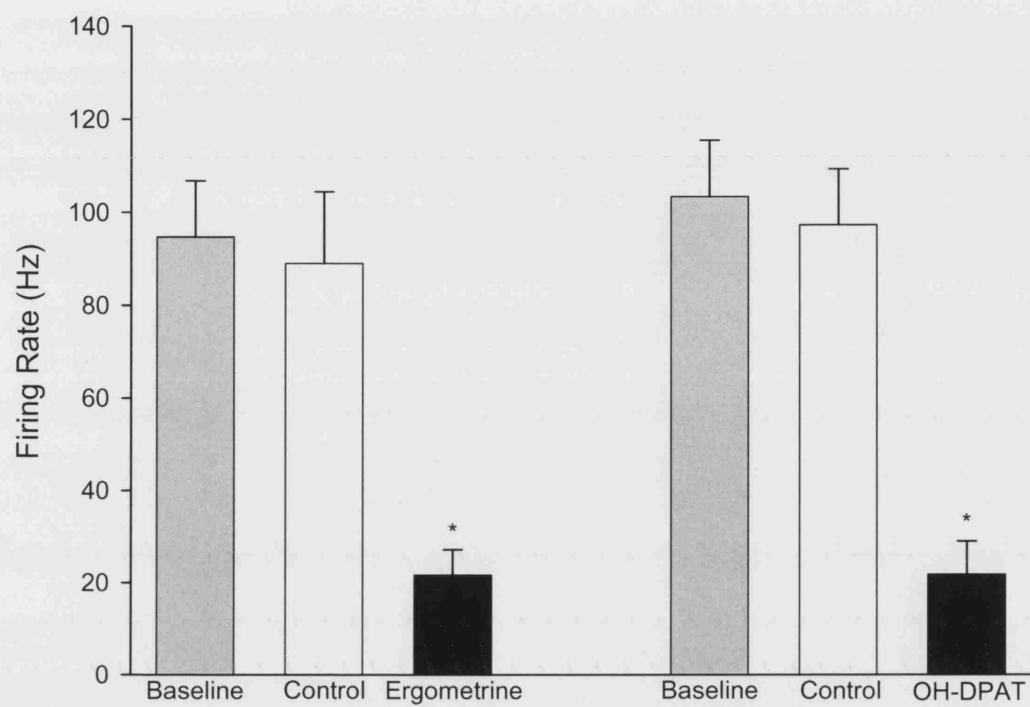


Figure 55: Inhibition of L-glutamate firing: ergometrine and (OH)-DPAT (pooled data)

Inhibitory effects of the serotonergic agonists ergometrine and (OH)-DPAT on L-glutamate neuronal firing rates (mean \pm SEM values are shown). (Ergometrine $n = 6$, OH-DPAT $n = 9$, * $P < 0.05$)

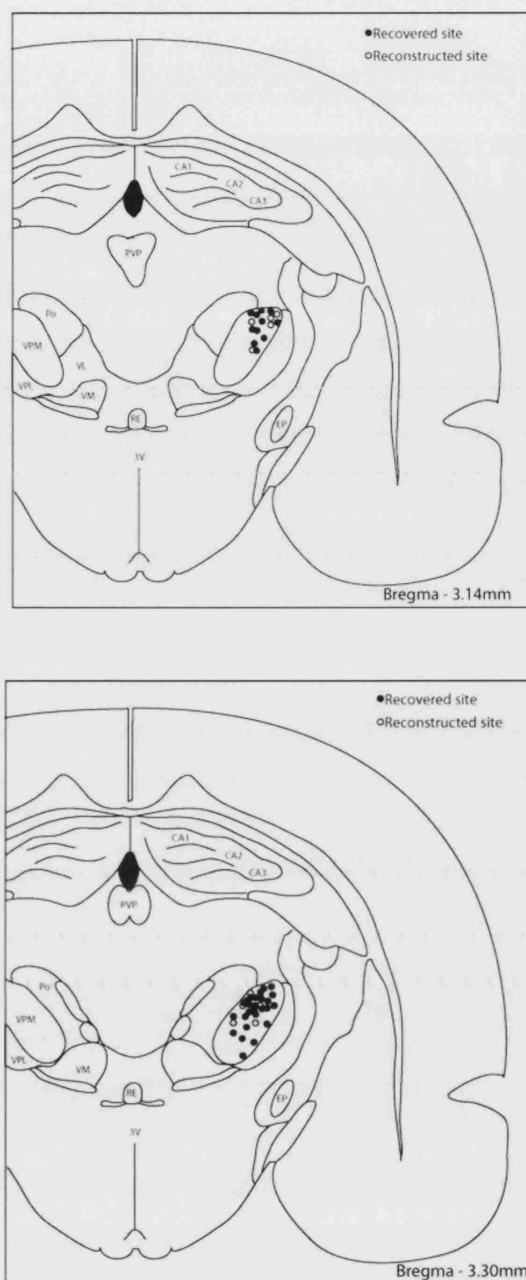


Figure 56: Histological confirmation of recording sites

Reconstruction of recording sites from lesions (filled circles) and by calculation (open circles) from microdrive readings. See Fig. 39 for details of the abbreviations used. Adapted from (Paxinos and Watson 1986).

Discussion

This study demonstrates that serotonergic neurotransmission in the VPM nucleus may potentially modulate trigeminovascular nociception. Naratriptan, a commonly used migraine abortive agent, is able to reversibly inhibit trigeminovascular nociceptive transmission in the thalamus through actions on 5-HT_{1A} and 5-HT_{1B/1D} receptors. Ergometrine had a comparable effect.

Modulation of trigeminovascular nociception by naratriptan

Naratriptan is a typical “second generation” triptan. It was selected for study because naratriptan is brain penetrant. It is thus likely that during a migraine attack naratriptan should be present in the human equivalent of the VPM following oral administration. Naratriptan was used as a representative example of a second generation triptan, however it may not be plausible to expect that all second generation triptans will behave in a similar fashion and this will require further investigation. Ergometrine maleate, and not the less hydrophilic dihydroergotamine, was used as a surrogate to study the effects of the ergot family on trigeminovascular nociceptive neurotransmission in the VPM because of its solubility.

Naratriptan reversibly inhibited the responses of thalamocortical relay neurons following SSS stimulation. Microiontophoretic ejection of naratriptan produced a proportionately smaller inhibition in comparison with an intravenous dose of 5mg/kg. This may reflect actions at other sites in the trigeminal pathway – for example the TNC (Storer and Goadsby, 1997) and also nerve endings in the dura in addition to a thalamic effect. The intravenous dose we have used is obviously considerably larger than that utilised in human practice. This may reflect the differential sensitivity of rat (r) and human (h) 5-HT_{1B/1D} receptors to triptans (Barnes and Sharp, 1999). Similar effects however have been observed using intravenous doses which are comparable to those employed in this study (Cumberbatch et al., 1998). Unfortunately as we do not know the concentration of naratriptan produced locally by microiontophoretic ejection this can only be speculation. The two methods of administration may have produced widely differing drug concentrations in the thalamus. If this were the case any discussion regarding the relative importance of the VPM versus extra-thalamic sites must be limited.

The scope of our studies examining the contributions of various 5-HT₁ receptor subtypes towards the actions of naratriptan is limited. Though relatively selective antagonists are now available for 5-HT_{1B} and 5-HT_{1D} receptors, they are not water soluble and therefore not suitable for microiontophoretic study. The selectivity of these compounds is also dependent on their concentration and this cannot be assured following microiontophoretic ejection. A selective water soluble 5-HT_{1B/1D} antagonist GR 127935 was used instead. Unfortunately this precludes any discussion on the relative contributions of 5-HT_{1B} and 5-HT_{1D} receptor agonism towards the effects of naratriptan.

The significant modulatory action resulting from 5-HT_{1A} receptor activation was surprising but not unexpected. All triptans have agonist properties at other members of the 5-HT₁ receptor subfamily - such as 5-HT_{1A} and 5-HT_{1F} receptors - though to varying degrees (Boers et al., 2000; Davidoff, 2002; Mitsikostas et al., 1999a). It cannot however be assumed that activation of 5-HT_{1A} and 5-HT_{1B/1D} receptors contribute equally to the modulatory actions of naratriptan. Though both antagonists had comparable effects, different concentrations of drug were used. GR 127935 also was less soluble than (S)-WAY 100135 and microiontophoretic ejection of GR 127935 was associated with higher failure rates (barrels generally had higher resistances and blocking occurred more frequently). The same limitations apply if comparisons of the inhibitory actions of naratriptan and OH-DPAT are attempted. Despite these caveats it appears that in the rat VPM naratriptan acts on - at the very least - 5-HT_{1B/1D} and 5-HT_{1A} receptors. It may be potentially useful to investigate possible actions of other 5-HT₁ receptors (notably 5-HT_{1F}) once suitable ligands are commercially available.

Ergot alkaloids have been used for the acute treatment of migraine for considerably longer than the triptans. Ergometrine maleate is not used for the treatment of migraine – though it is used in obstetric practice. It is however more water soluble than either ergotamine or dihydroergotamine (DHE) making it more suitable for microiontophoretic ejection. The pharmacological actions of ergometrine are similar to DHE. Both compounds bind to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT₂, α_1 and α_2 adrenoceptors and D₂ dopamine receptors (Silberstein, 1997). Ergometrine was also able to inhibit thalamocortical firing following L-glutamate ejection. Unfortunately when studying the effect of a drug on the response to SSS stimulation it must be ejected for relatively prolonged periods – on average between 1 and 4 minutes.

Invariably the resistance of the barrel containing ergometrine was high ($\sim 150\text{ M}\Omega$) so “blocking” occurred on all occasions when this prolonged ejection was attempted. In our other experiments in which modulation of both responses was studied, efficacy in one paradigm was matched by a similar response in the other. Unfortunately as inhibition of the response to SSS stimulation was not demonstrated, we cannot unequivocally assume that ergometrine is capable of inhibiting nociceptive neurotransmission. We can only say at present that ergotamine, and probably other ergot derivatives, inhibits L-glutamate evoked firing in VPM neurons activated a nociceptive stimulus applied to an intra-cranial vascular structure which is innervated by the trigeminal nerve.

In conclusion these results suggest that naratriptan may be able to modulate the flow of trigeminovascular sensory information through the VPM. It appears to do so by agonism at both 5-HT_{1A} and $5\text{-HT}_{1B/1D}$ receptors. Ergometrine maleate - possibly through serotonergic mechanisms – probably also inhibits trigeminal nociceptive transmission in the VPM. The thalamus may therefore be an additional site where triptans exert their therapeutic effect in migraine.

Study 3: Investigation of the modulatory actions of propranolol on trigeminovascular neurotransmission by thalamocortical neurons of the rat VPM nucleus.

Introduction

The β -adrenergic blockers are perhaps the most commonly prescribed migraine preventives in current clinical practice. Little is known however of where or how they act. Given that microiontophoresis of β -adrenergic blockers has been reported to have an inhibitory action on thalamic neurons, this study investigated whether propranolol may have an action on thalamocortical relay cells responding to SSS stimulation. The effects of propranolol on the responses of thalamocortical neurons following SSS stimulation and L-glutamate ejection were studied. Propranolol is a non-selective β -adrenoceptor antagonist. It also has membrane stabilising properties and binds with relatively high affinity to 5-HT_{1A} and 5-HT_{1B} receptors. The pharmacological basis for propranolol's action in this model was examined using selective β -receptor antagonists. Table 28 summarises the drugs used in this study.

Table 28: Summary of drugs used in Study 3

Drug	Pharmacological action	Ejection Polarity	Control
Propranolol	β -adrenoceptor antagonist	(+)	Na ⁺ , H ⁺
Atenolol	β_1 -adrenoceptor antagonist	(+)	Na ⁺ , H ⁺
ICI 118,551	β_2 -adrenoceptor antagonist	(+)	Na ⁺ , H ⁺
SR 59230A	β_3 -adrenoceptor antagonist	(+)	Na ⁺ , H ⁺
Isoproterenol	β -adrenoceptor agonist	(+)	Na ⁺ , H ⁺
Procaterol	β_2 -adrenoceptor agonist	(+)	Na ⁺ , H ⁺

(+): cation

Results

A total of 63 cells were studied (Table 29). Cells responded with an increased probability of firing to electrical SSS stimulation with a mean response probability of $80 \pm 2\%$ (mean \pm S.E.M). The results are presented in detail in Tables 30 to 32. The recording sites are shown in Fig. 67.

Table 29: Receptive field characteristics of the neurons in Study 3

Receptive Field Characteristics	Number of cells
Low threshold mechanosensitive (LTM)	7
Nociceptive specific (NS)	1
Wide dynamic range (WDR)	5
Vibrissal (no. of vibrissae: mean \pm SEM)	50 (2.8 \pm 0.2)

Effects of propranolol

Propranolol was able to reversibly reduce the probability of thalamocortical neuron firing in response to electrical stimulation of the SSS ($n = 13$, $P < 0.001$) in comparison to control ejection of Na^+/H^+ ions (Fig. 55 and 57). Similarly it was able to inhibit the response of relay neurons to ejection of L-glutamate ($n = 12$, $P < 0.001$) (Fig. 61 and 63). The inhibition was often prolonged (6-10 minutes) but reversible. The effects of propranolol on spontaneous neuronal activity were not specifically studied.

This inhibitory effect appeared to be due largely to β -adrenoceptor antagonist properties of propranolol because the inhibition could be reduced by concurrent ejection of the β -agonist isoproterenol. The reduction in firing probability in response to SSS stimulation was significantly attenuated ($n = 7$, $P = 0.02$) in comparison to propranolol alone (Fig. 56 and 58). The neuronal firing rate in response to L-glutamate was also increased by co-ejection of isoproterenol ($n = 5$, $P = 0.006$) with propranolol. Isoproterenol itself did not produce a significant increase in neuronal firing in either of the two experiments ($P = 0.4$, $P = 0.6$; Fig. 62 and 64). Though propranolol antagonises 5-HT_{1A} and $5\text{-HT}_{1B/1D}$ receptors, it should be recalled that antagonism with the selective antagonists (S)-WAY 100135 and GR127935 did not significantly inhibit the response to L-glutamate (Page 153). This also supports a β -adrenergic basis for propranolol's inhibitory action.

Selectivity of inhibition produced by β -receptor antagonists

The β_1 receptor antagonist atenolol was able to inhibit both responses in a similar fashion to propranolol (SSS: $n = 6$, $P = 0.003$; L-glutamate: $n = 9$, $P < 0.001$; Fig. 59 and 60, 65 and 66). The selective β_2 antagonist ICI 118,551 however was not able to produce any inhibition in comparison to ejection of Na^+ (SSS: $n = 6$, $P = 0.9$; L-glutamate: $n = 7$, $P = 0.4$). Given the low solubility of ICI 118,551, to ensure that a possible action of β_2 receptors was not missed we studied the effects of microiontophoresis of the potent β_2 agonist procaterol. This again had no effect on the response to SSS stimulation ($t_6 = 0.5$, $P = 0.9$) or L-glutamate ejection ($n = 6$, $F_{1,4} = 0.01$, $P = 0.9$). SR 59230A, a selective β_3 receptor antagonist, also had no demonstrable effect on the response probability following SSS stimulation ($n = 6$, $P = 0.7$) or firing rate resulting from L-glutamate ejection ($n = 4$, $P = 0.2$) (Fig 66).

Table 30: Effects of β -adrenergic antagonists on the response following SSS stimulation.

i) Mean response probabilities following SSS stimulation

β Adrenoceptor Antagonist	Baseline	Control (Na^+ , H^+ ions)	Drug
Propranolol	$69 \pm 4\%$	$76 \pm 6\%$	$39 \pm 4\%^*$
Atenolol	$84 \pm 9\%$	$80 \pm 10\%$	$42 \pm 7\%^*$
ICI 118551	$94 \pm 5\%$	$94 \pm 7\%$	$91 \pm 5\%$
SR 59230A	$91 \pm 8\%$	$88 \pm 8\%$	$86 \pm 10\%$

Response probabilities (%) of thalamocortical neurons following electrical stimulation of the SSS. When a neuron was located a series of 50 stimuli were delivered to the SSS and the response probability (as a percentage) calculated. Results under the three test conditions were pooled and are presented as the mean \pm SEM (* P was calculated < 0.05).

ii) Statistical results

β Adrenoceptor Antagonist	Drug v. Control	Drug v. Baseline	Baseline v. Control
Propranolol	$t_{12} = 9$, $P < 0.001$	$t_{12} = 9.8$, $P < 0.001$	$t_{12} = 1.7$, $P = 0.1$
Atenolol	$t_5 = 5.3$, $P = 0.003$	$t_5 = 7.9$, $P = 0.001$	$t_5 = 2.1$, $P = 0.09$
ICI 118551	$t_5 = 0.2$, $P = 0.9$	$t_5 = 2.1$, $P = 0.09$	$t_5 = 0.8$, $P = 0.4$
SR 59230A	$t_5 = 0.4$, $P = 0.7$	$t_5 = 1.3$, $P = 0.3$	$t_5 = 0.8$, $P = 0.5$

Paired sample t -test analysis of the effects of β -adrenergic antagonists on the response probabilities of thalamocortical neurons following electrical stimulation of the SSS.

Table 31: Effects of β -adrenergic antagonists on the L-glutamate evoked neuronal response.

i) Mean firing rates in response to L-glutamate ejection

β Adrenoceptor Antagonist	Baseline	Control (Na ⁺ , H ⁺ ions)	Drug
Propranolol	46 \pm 6	46 \pm 10	13 \pm 5*
Atenolol	98 \pm 12	81 \pm 13	30 \pm 8*
ICI 118551	62 \pm 13	40 \pm 9	42 \pm 9
SR 59230A	85 \pm 20	69 \pm 15	83 \pm 18

Action potential firing rates (Hz) of third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result \pm SEM (* P was calculated < 0.05).

ii) Statistical results

β Adrenoceptor Antagonist	Drug v. Control	Baseline v. Control
Propranolol	$n = 12$, $F_{1,4} = 38$, $P < 0.001$	$n = 12$, $F_{1,2.5} = 0.05$, $P = 0.95$
Atenolol	$n = 9$, $F_{1,4} = 46$, $P < 0.001$	$n = 9$, $F_{1,1.8} = 3$, $P = 0.1$
ICI 118551	$n = 7$, $F_{1,4} = 0.8$, $P = 0.4$	$n = 7$, $F_{1,4} = 5$, $P = 0.06$
SR 59230A	$n = 4$, $F_{1,1.2} = 0.2$, $P = 0.2$	$n = 4$, $F_{1,1.3} = 8$, $P = 0.07$

F and P values calculated for each treatment group comparing the effects of beta-adrenergic antagonists and control during co-ejection with L-glutamate. The effect of control ejection on the baseline response was also studied.

Table 32: Antagonism of the actions of propranolol on the L-glutamate and SSS stimulation response by isoproterenol.

i) Mean firing rates in response to L-glutamate ejection and mean response probabilities following SSS stimulation

Neuronal Response	Baseline	Isoproterenol	Propranolol	Propranolol + Isoproterenol
a) L-Glutamate microiontophoresis	94 ± 16	87 ± 8	22 ± 2*	57 ± 6#
b) Electrical SSS stimulation	78 ± 5%	70 ± 4%	40 ± 7%*	68 ± 6%#

a) Action potential firing rates (Hz) of third order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean result ± SEM.

b) Response probabilities (%) of thalamocortical neurons following electrical stimulation of the SSS. When a neuron was located a series of 50 stimuli were delivered to the SSS and the response probability (as a percentage) calculated. Results under the three test conditions were pooled and are presented as the mean ± SEM (** P was calculated < 0.05, * relative to the baseline response, # relative to the propranolol response).

ii) Statistical results

Neuronal Response	Propranolol v. Baseline	Isoproterenol v. Baseline	Propranolol + Isoproterenol v. Propranolol	Propranolol + Isoproterenol v. Baseline
a) L-Glutamate microiontophoresis	$n = 5$, $F_{1,4} = 21$, $P = 0.01$	$n = 5$, $F_{1,4} = 0.4$, $P = 0.6$	$n = 5$, $F_{1,4} = 28$, $P = 0.006$	$n = 5$, $F_{1,4} = 8$, $P = 0.05$
b) Electrical SSS stimulation	$n = 7$, $t_6 = 8.0$, $P < 0.001$	$n = 7$, $t_6 = 0.8$, $P = 0.4$	$n = 7$, $t_6 = 3.0$, $P = 0.02$	$n = 7$, $t_6 = 1.5$, $P = 0.2$

a) F and P values calculated for each treatment group comparing the effects of propranolol ± isoproterenol during co-ejection with L-glutamate.

b) Paired sample t -test analysis of the effects of propranolol, isoproterenol and propranolol + isoproterenol.

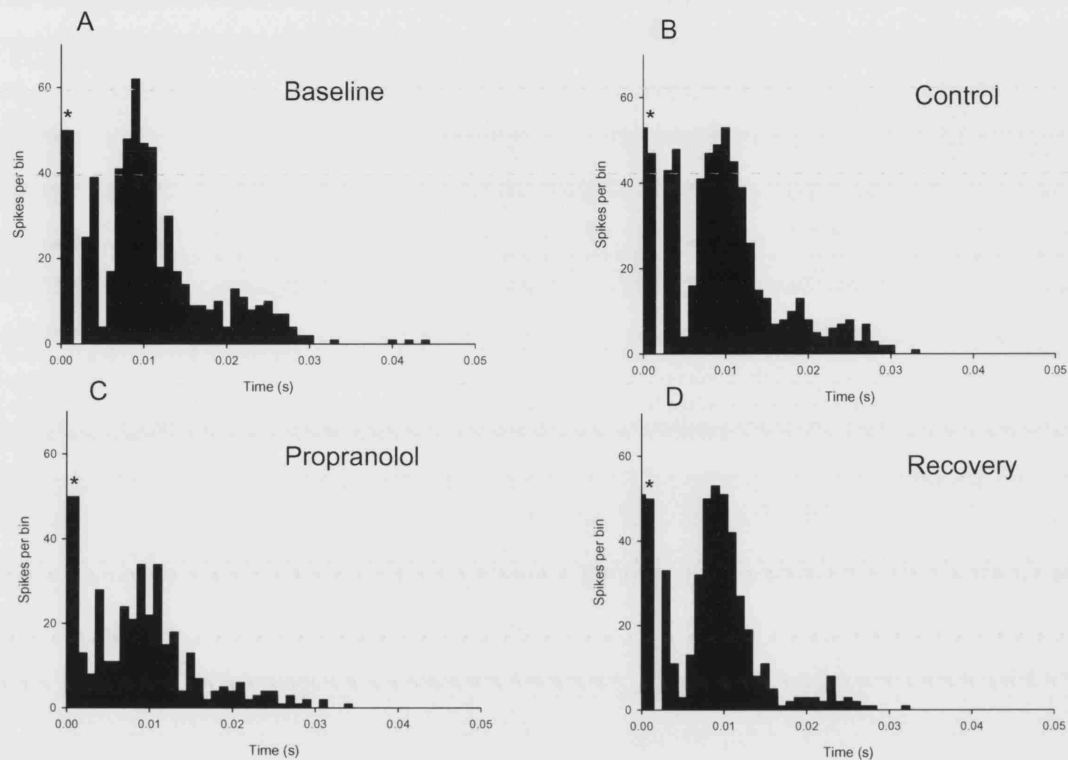


Figure 57: SSS stimulation: effects of propranolol

Post-stimulus histograms demonstrating that propranolol reversibly inhibits the response of this third order relay neuron following SSS stimulation. The inhibitory effect of propranolol ejection in comparison to the control ejection of Na^+ and H^+ ions is clearly demonstrated.

(*Stimulus artefact)

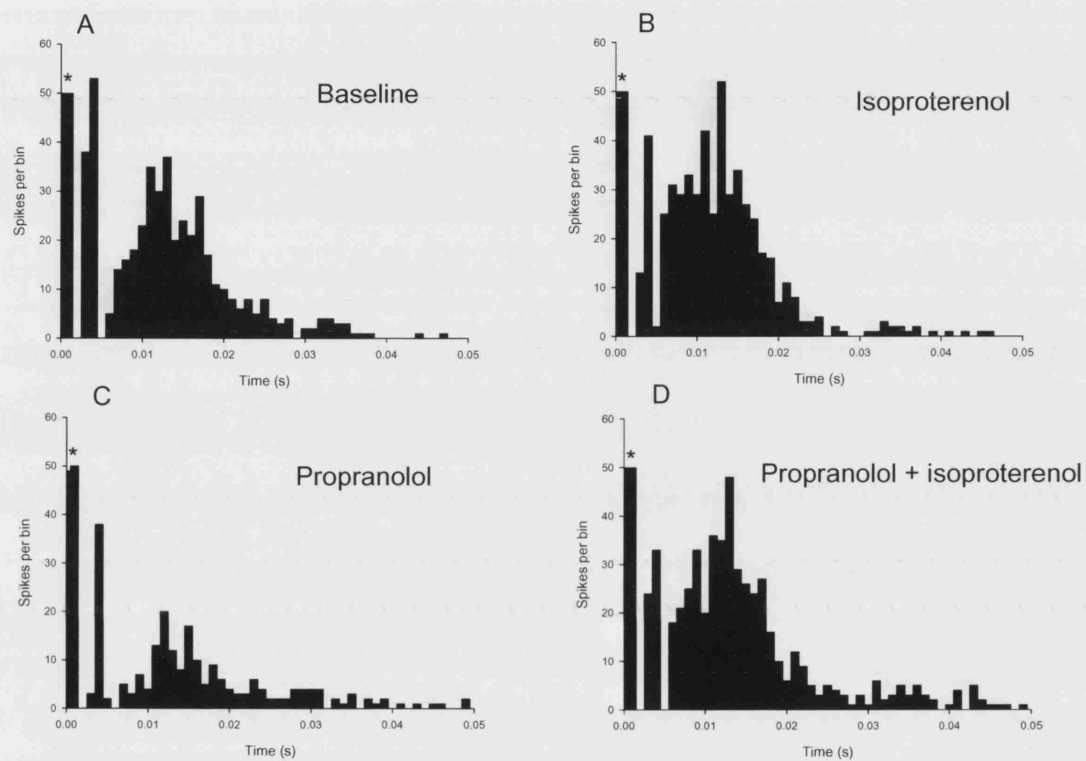


Figure 58: SSS stimulation: effects of selective β -adrenoceptor antagonism

Propranolol reversibly inhibited the response of this third order relay neuron following SSS stimulation. Co-ejection of the non-specific β -agonist isoproterenol was able to prevent this, suggesting that propranolol's β -antagonism is responsible for this action, and not necessarily its membrane stabilising properties or antagonism of 5-HT₁ receptors.

(*Stimulus artefact)

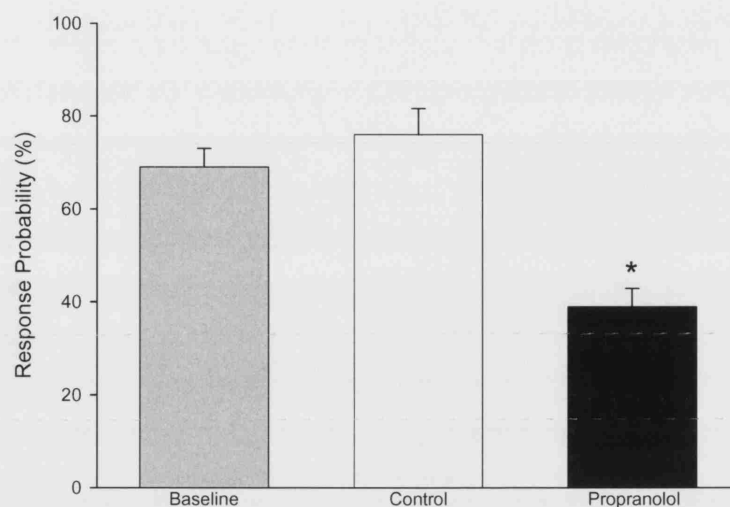


Figure 59: SSS stimulation: effects of propranolol (pooled data)

Comparison of the mean (\pm SEM) response probabilities of VPM neurons following SSS stimulation. Propranolol significantly inhibited this response. ($n = 13$, * $P < 0.05$)

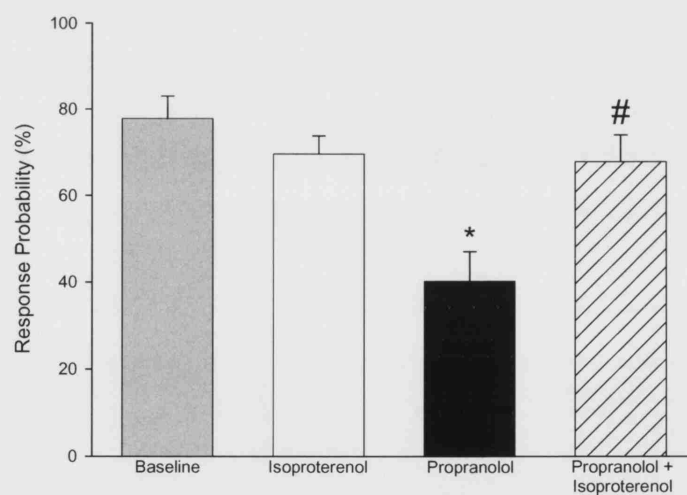


Figure 60: SSS stimulation: effects of selective β -adrenoceptor antagonism (pooled data)

Co-ejection of isoproterenol was able to antagonise the inhibitory effects of propranolol on the SSS response (mean \pm SEM). ($n = 7$, * # $P < 0.05$; * relative to control, # relative to propranolol response)

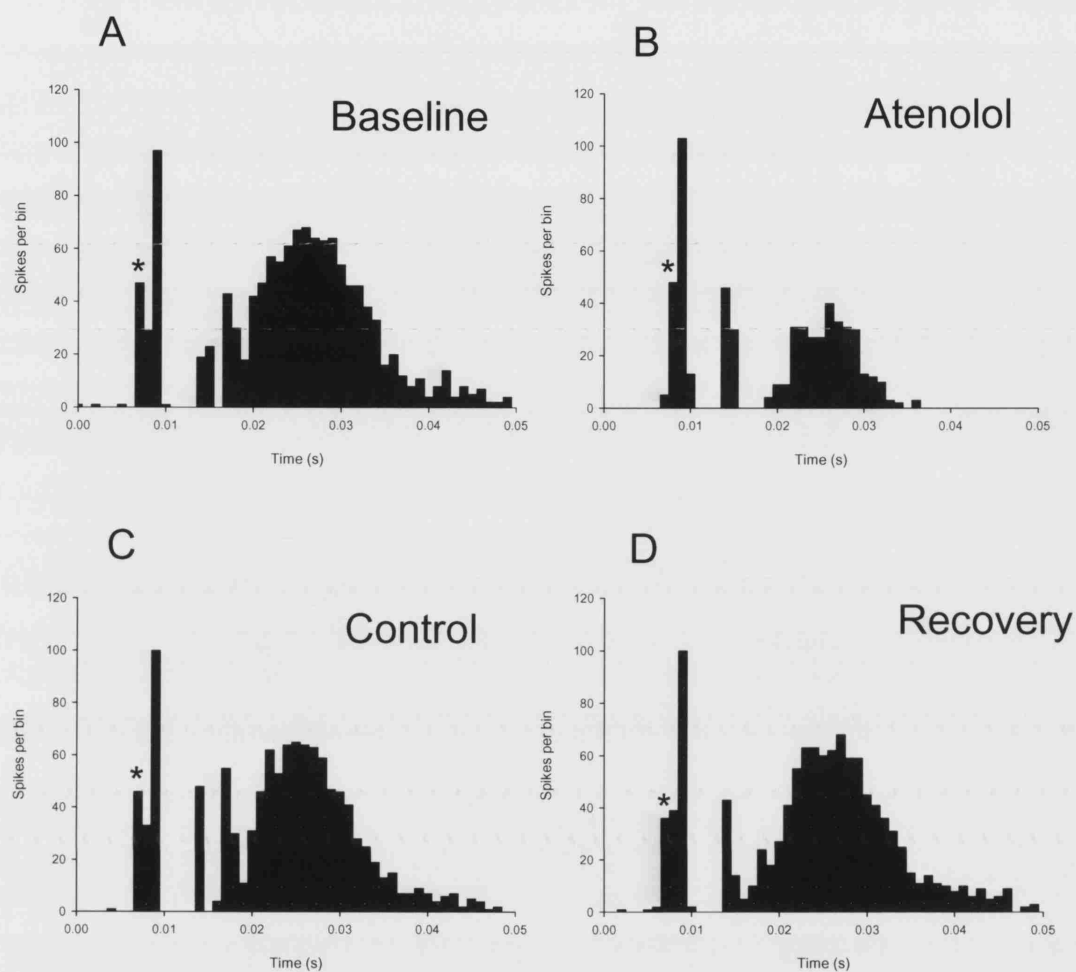


Figure 61: SSS stimulation: selective β_1 -adrenoceptor antagonism

Post-stimulus histograms demonstrating the response following microiontophoretic ejection of the selective β_1 antagonist atenolol (* stimulus artefact).

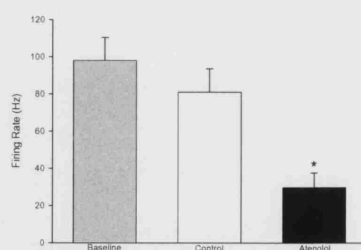


Figure 62: SSS stimulation: selective β_1 -adrenoceptor antagonism (pooled data)

Comparison of the effects of ejection of control and atenolol on the mean (\pm SEM) response probabilities of thalamocortical neurons following electrical stimulation of the SSS. ($n = 6$, * $P < 0.05$)

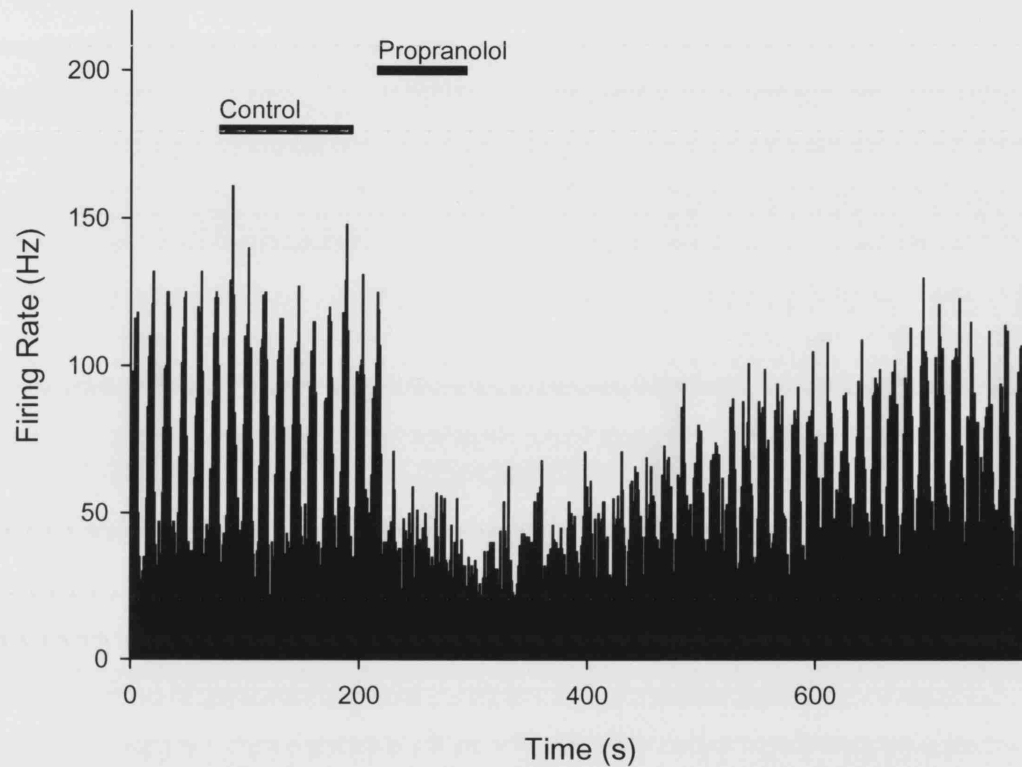


Figure 63: L-glutamate firing: inhibition by propranolol

Effects of propranolol on the firing rate of a third order relay neuron following ejection of L-glutamate. The effects of propranolol and control (Na^+ and H^+ ions) – both ejected at the same ejection current (55nA) and for approximately the same duration (70 and 100s respectively) – were compared. Propranolol clearly inhibited L-glutamate evoked neuronal firing.

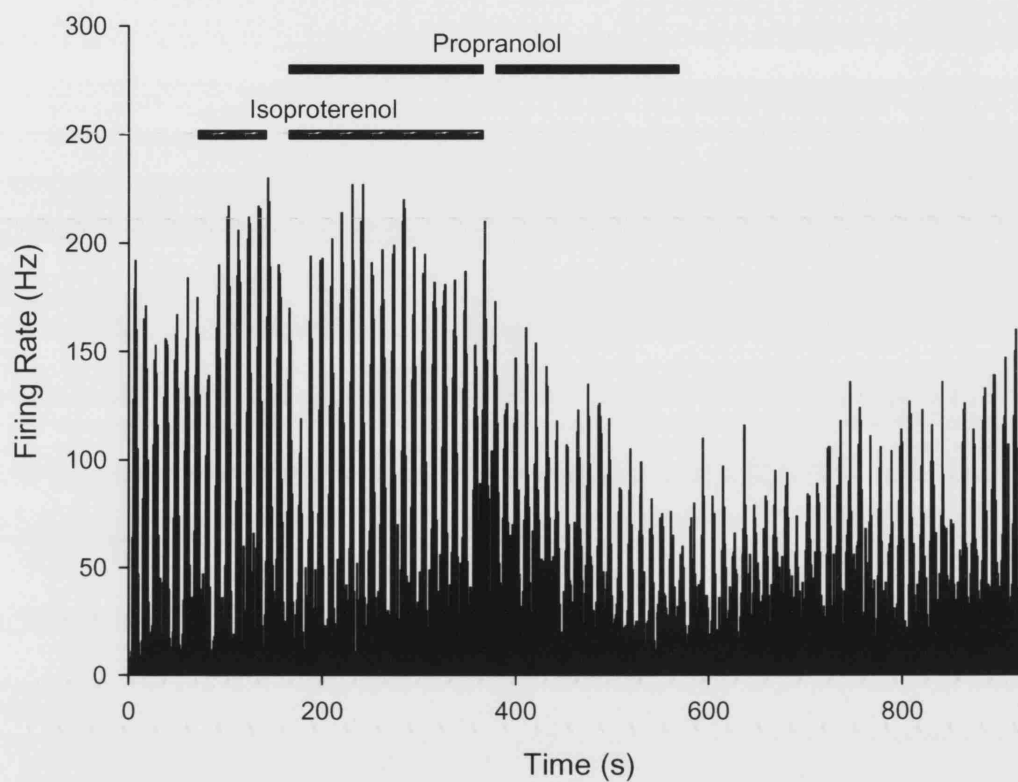


Figure 64: L-glutamate firing: β -adrenoceptor antagonism

Antagonism of propranolol's inhibition of the L-glutamate evoked response by co-ejection of isoproterenol. In this example propranolol produced little inhibition when isoproterenol was co-ejected – isoproterenol could only partially antagonise the effects of propranolol. When propranolol was ejected a second time, at the same ejection current and for the same duration, without isoproterenol the inhibition was marked.

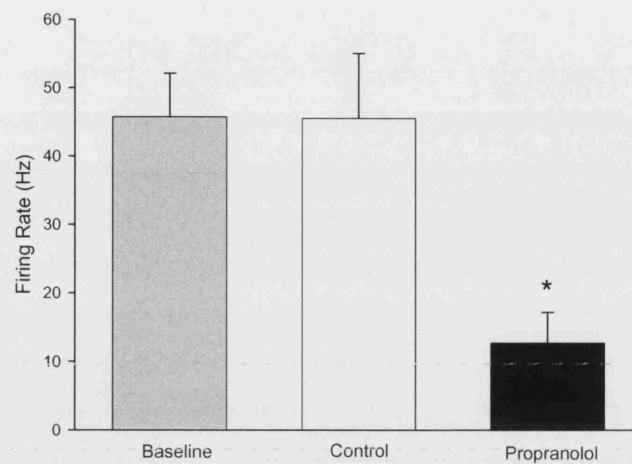


Figure 65: L-glutamate firing: inhibition by propranolol (pooled data)

Comparison of the effects of drug ejection on the mean (\pm SEM) firing rates (Hz) of thalamocortical neurons during five second ejection pulses of L-glutamate. The effects of propranolol and control ejection are compared. ($n = 12$, * $P < 0.05$)

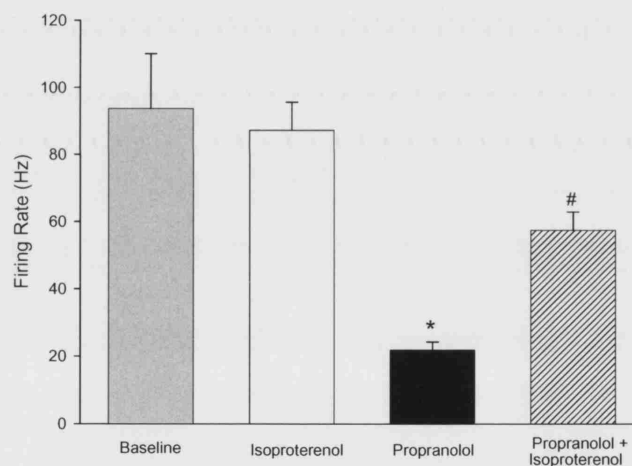


Figure 66: L-glutamate response: β -adrenoceptor antagonism (pooled data)

When the mean (\pm SEM) responses of neurons within the group were calculated, isoproterenol was clearly able to partially antagonise the inhibitory effects of propranolol on the L-glutamate response. ($n = 5$, * $P < 0.05$; # relative to the control, # relative to propranolol)

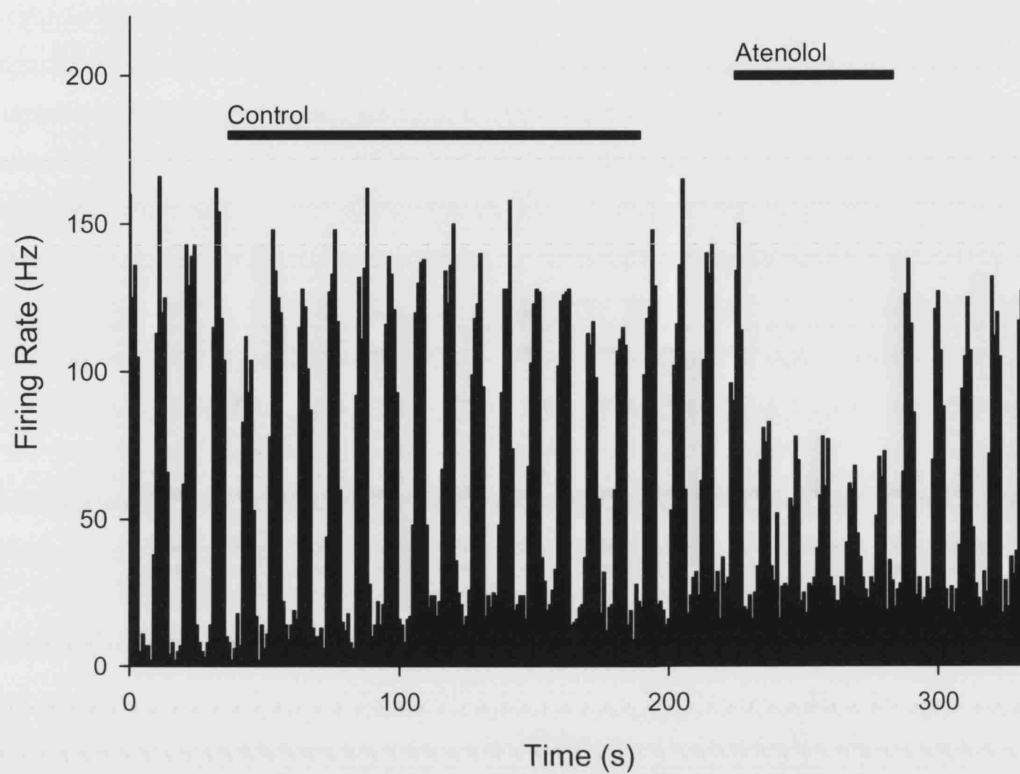
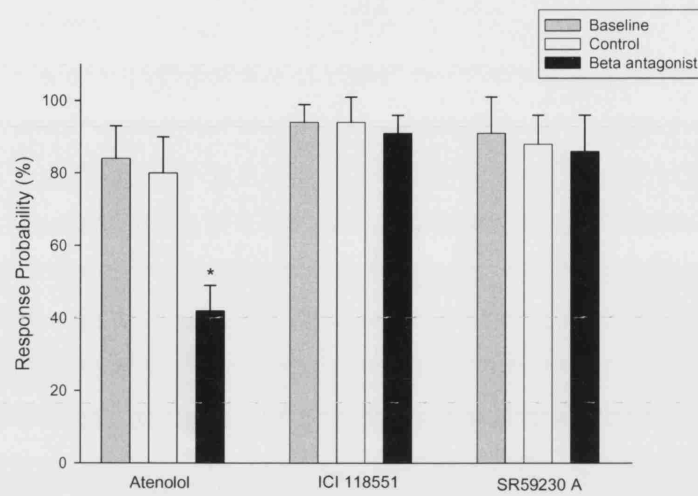


Figure 67: Selective β_1 -receptor antagonism of L-glutamate firing

Inhibitory actions of the selective β_1 -antagonist atenolol. β_2 - and β_3 -antagonists did not demonstrate any effect. Atenolol is not an aryloxyalkylamine β -blocker and so has low affinity for rat and human 5-HT_{1A} and r5-HT_{1B} receptors.

a)



b)

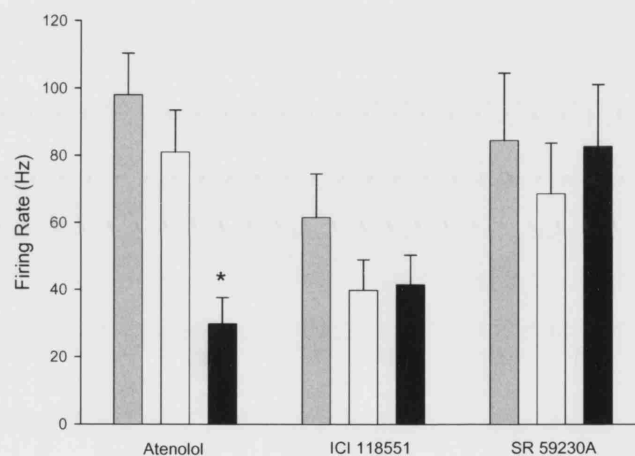


Figure 68: Selective β -adrenoceptor antagonism: effects on SSS and L-glutamate responses

Comparison of the effects of selective β -adrenoceptor antagonists (β_1 : atenolol, β_2 : ICI 118551, β_3 : SR59230A) on: (a) the responses of thalamocortical cells following electrical stimulation of the SSS ($n = 6$) and (b) ejection of L-glutamate relative to control and baseline responses (Atenolol $n = 9$, ICI 118551 $n = 7$, SR 59230A $n = 4$, * $P < 0.05$)

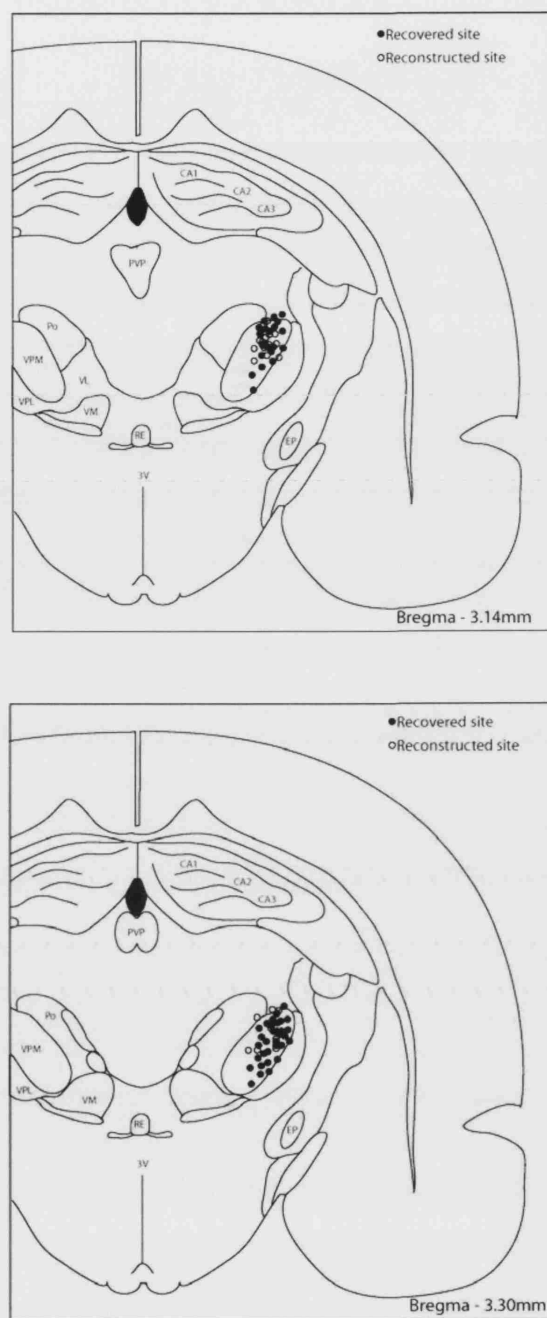


Figure 69: Histological confirmation of recording sites

Reconstruction of recording sites from lesions (filled circles) and by calculation (open circles) from microdrive readings. See Fig. 39 for details of abbreviations. Adapted from (Paxinos and Watson 1986).

Discussion

The data demonstrate a robust and reproducible inhibitory effect for β_1 -adrenoceptor antagonist activity in ventroposteromedial thalamic neurons responding to nociceptive trigeminovascular input. There is no effect of β_2 or β_3 adrenoceptor antagonists in our model. These data offer a plausible locus of activity for propranolol via β_1 receptor antagonism in the trigeminovascular pain pathway, and a reasonable basis for considering the thalamus as a possible target for preventive treatments in migraine. In this context it is noteworthy that models of neurogenic dural vasodilation and plasma protein extravasation have failed to demonstrate a peripheral action for propranolol, which makes a central action even more attractive (Akerman et al., 2001; Markowitz et al., 1988).

Local ejection of propranolol by microiontophoresis was able to inhibit the response following superior sagittal sinus stimulation. Furthermore, propranolol was also able to modulate the response of third order neurons to L-glutamate, indicating a probable post-synaptic site of action. This inhibition was reversible and had a prolonged time course ranging from 6 to 10 minutes. Unfortunately we did not examine the effect of propranolol on spontaneous neuronal firing so no conclusions may be drawn regarding tonic catecholaminergic influences on VPM neurons.

Concurrent ejection of isoproterenol, a non-specific β -agonist, partially antagonised the inhibitory actions of propranolol on the responses to both SSS stimulation and L-glutamate ejection. There are two possible explanations for this: a) propranolol has multiple mechanisms of action not solely restricted to antagonism of β -adrenoceptors or b) propranolol has a purely β -adrenergic action but there was insufficient antagonism of its actions by isoproterenol. Propranolol - like other aryloxyalkylamines - binds with high affinity to rat 5-HT_{1A/1B/1D} receptors (Nishio et al., 1989). It also has membrane stabilising properties. Ejection of the selective 5-HT_{1A} and 5-HT_{1B/1D} antagonists however had no appreciable effects on the response of neurons to ejection of L-glutamate in Study 2 (Page 153). This would suggest that technical limitations resulted in insufficient quantities of isoproterenol being delivered to the desired receptors to antagonise completely the effects of propranolol. It seems likely therefore that propranolol is modulating these neuronal responses through actions on β -adrenoceptors. Isoproterenol was more effective at antagonising the inhibitory actions of propranolol on the responses following SSS stimulation than L-glutamate ejection. It was noted that when multiple barrels are used simultaneously

for drug ejection the risk of barrel blocking increased. This was less of a problem when only two barrels were used eg isoproterenol and propranolol - in studies looking at the response to SSS stimulation, however with three barrels it occurred frequently. Another problem was neuronal suppression at high ejection currents. This problem was particular to modulation of the L-glutamate response. As the ejection current of isoproterenol was increased, the combined currents of isoproterenol and propranolol could suppress neuronal firing due to L-glutamate ejection. The same effect could be observed with the ejection of control at sufficiently high currents ($> 100\text{nA}$). Somewhat surprisingly the ejection of isoproterenol itself did not result in an increase in the response of VPM neurons to either stimulus. This unfortunately does call into question whether propranolol is genuinely exerting its inhibitory action through β -adrenoceptors. In order to clarify the action of propranolol several further studies should ideally have been performed. The first would have investigated whether there is a tonic adrenergic input to the VPM nucleus. If there is, propranolol should have been expected to have an inhibitory effect on the resting firing rates of VPM neurons (it could be argued that isoproterenol would not necessarily have an effect if the resting adrenergic tone was high under our experimental conditions). Another would test whether concurrent ejection of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} antagonists could block the effects of propranolol. We have not excluded the possibility that the inhibitory effect of propranolol is through an action at these receptors and this remains a possibility (though see below).

Further results however tend to confirm that the inhibitory actions of β -blockers are mediated by antagonism of β -adrenoceptors, specifically the β_1 type. This is supported by the actions of the selective β_1 antagonist atenolol. Unlike the aryloxyalkylamine β -blockers, such as propranolol, atenolol has low affinity for 5-HT₁ receptors (Nishio et al., 1989). It also lacks membrane stabilising activity. Atenolol had similar actions to propranolol, reversibly inhibiting both neuronal responses. This would suggest that the effect may be a specific action of β_1 receptor antagonism as the β_2 receptor antagonist (ICI 118551) and β_3 receptor antagonist (SR59230 A) produced no appreciable inhibition. ICI 118551 is poorly soluble in water and the concentration of ICI 118551 (0.01M) was significantly less than the other β -antagonists (propranolol: 0.1M, atenolol: 0.025M and SR 59230A: 0.05M). Though no effect was seen following ejection of the potent β_2 agonist procaterol, a role for β_2 -adrenoceptors in this model is not totally excluded. As isoproterenol had no over-all effect on neuronal

responses, it is not surprising that procaterol behaved in a similar fashion. This experiment should be repeated when a more soluble β_2 antagonist becomes available. It is tempting to suggest that β_1 antagonists inhibit the inward rectifying current I_h (McCormick and Pape, 1990), preventing neuronal repolarisation and therefore repetitive firing. Input from the locus coeruleus facilitates the transmission of sensory information from the VPM to the cortex as neurons are capable of rapid firing rates. It could be argued that β -blockers would not necessarily have an inhibitory effect on resting neuronal firing rates if they were discharging slowly. If we accept this argument we should however have seen an increase in neuronal firing (in response to either SSS stimulation or L-glutamate ejection) when the non-selective β -agonist isoproterenol was ejected. It would be interesting to investigate the effects of a potent and selective β_1 agonist. If such an agent could facilitate neuronal firing it would provide supportive evidence for our proposed mechanism of action. The fact that atenolol had a similar effect to propranolol makes it less likely however that serotonergic and local anaesthetic actions are responsible for this inhibition. We still however cannot exclude the possibility that some other non β_1 -mediated mechanism is responsible for the observed inhibition.

Study 4: Investigation of the role of high threshold voltage gated calcium channels (P/Q-, L- and N-type) in trigeminovascular nociceptive neurotransmission in the trigeminocervical complex of the cat.

Introduction

Migraine is a common condition and molecular genetics have recently offered new insights to its pathogenesis. Familial Hemiplegic Migraine (FHM) is a rare variant of migraine with aura that is transmitted in an autosomal dominant fashion. Several of the causative mutations of this condition have been identified in the CACNA1A gene on chromosome 19 (Ophoff et al., 1998). This gene encodes the α_{1A} ($Ca_v2.1$) subunit of the P/Q high threshold voltage dependent calcium channel (VDCC). The FHM locus has also been linked to migraine both with and without aura, suggesting that calcium channelopathies may contribute in part to the pathogenesis of migraine (Goadsby et al., 2002b). Disorders of calcium ion flux specifically related to abnormalities in VDCC have been implicated in the pathogenesis of several other neurological conditions including specific varieties of epilepsy and cerebellar ataxia (Kullmann, 2002; Kullmann and Hannah, 2002; Ophoff et al., 1998).

VDCCs mediate the influx of calcium ions into cells in response to depolarisation of the cell membrane (Catterall, 2000). Each channel is a heteromeric assembly of up to four subunits. VDCCs have been classified according to electrophysiological and pharmacological properties. Using one classification system six subtypes are recognised: P, Q, L, N, R and T (Tsien et al. 1988). An alternative classification is based on molecular biological studies of the sequence homology between the cloned isoforms of the α_1 subunit, identifying 10 subtypes (Ertel et al., 2000). P-, Q-, L-, N- and R-type VDCCs are characterised by having a higher threshold of activation than T channels (Page 83). While L- and T-type VDCCs have a widespread distribution amongst many cell types, P/Q-, N- and R-type channels are largely confined to neurons. High threshold VDCCs are one of the principal means of calcium influx into neurons. Calcium ions are perhaps unique in that they regulate not only neuronal excitability as charge carriers, but also act as second messengers affecting many biochemical processes within the nerve cell.

This study investigated the contribution of post-synaptic high threshold VDCCs towards action potential generation in the cell bodies of second order neurons of the trigeminocervical complex (TCC). Electrical stimulation of the superior sagittal sinus (SSS) was used to identify afferent intracranial nociceptive neurons. Action potentials were triggered in SSS-activated cells by microiontophoretic ejection of the excitatory

neurotransmitter L-glutamate directly onto the cell body of the second order neurons. The ability of both selective and non-selective blockers of VDCC to inhibit action potential firing was studied. The drugs used are summarised in Table 33.

Table 33: Summary of drugs used in Study 4

Drug	Pharmacological Action	Ejection Polarity	Control
Cadmium	Non-selective VDCC blocker	(+)	Na ⁺
ω -Agatoxin IVa	P/Q-type VDCC blocker	(+)	Na ⁺ , H ⁺
ω -Agatoxin TK	P/Q-type VDCC blocker	(+)	Na ⁺ , H ⁺
ω -Conotoxin GV1a	N-type VDCC blocker	(+)	Na ⁺ , H ⁺
Calciseptine	L-type VDCC blocker	(+)	Na ⁺ , H ⁺

(+): cation

Results

Extracellular recordings were made from 46 neurons in the trigeminocervical complex (Table 34). Cells were located ± 3 mm to the midpoint of the C₂ rootlets at a depth of between 1000 – 3600 μ m from the dorsal surface of the cord. Cells responded with an increased probability of firing following electrical SSS stimulation with latencies consistent with A δ fibres (latency 9 – 12 ms, velocity 2.9 – 3.8 ms⁻¹). The inhibition produced by all the peptide blockers was reversible and the time course of this reversibility was short. This effect was consistently observed for all peptides and the implications of this observation are explored in the discussion. The mean responses (\pm SEM) of neurons in the different treatment groups, and statistical analyses are displayed in Table 35 while recording sites are shown in Fig. 78.

Table 34: Receptive field characteristics of the neurons in Study 4

Receptive Field Characteristics	Number of cells
Low threshold mechanosensitive (LTM)	8
Nociceptive Specific (NS)	7
Wide dynamic range (WDR)	31

Effect of non-selective high threshold VDCC blockade

Cadmium ions were used to non-selectively block all high threshold VDCCs. The response to ejection of L-glutamate was significantly inhibited by ejection of cadmium ions in comparison to ejection of sodium ions ($n = 5$, $P = 0.01$). There was no statistical difference between the baseline and control response (Fig. 68 and 69).

P/Q-type channels

Two types of ω -agatoxin were employed, TK and IVa. ω -Agatoxin IVa was associated with higher rates of channel “blocking” - hence the use of ω -agatoxin TK and the smaller numbers in the IVa group, though TK is less well characterised than ω -agatoxin IVa. A total of 26 cells (4 LT, 4 NS, and 18 WDR) were studied; three cells were studied using both toxins. We found that cell firing evoked by microiontophoretic application of L-glutamate was significantly inhibited by both ω -agatoxin IVa ($n = 10$, $P = 0.007$) and ω -agatoxin TK ($n = 19$, $P < 0.001$). The effect was reversible and, as with cadmium, there was no statistical difference between the baseline response and control (Fig. 70 to 73).

N-type channels

ω -Conotoxin GVIA, a blocker of N-type VDCCs, inhibited cells activated by L-glutamate in a reversible manner in comparison with the ejection of control ($n = 13$, $P < 0.001$). The control ejection did not produce a significant inhibition (Fig. 74 and 75).

L- type channels

The L-type VDCC blocker calciseptine significantly inhibited the L-glutamate response ($n = 8$, $P < 0.001$). In these experiments the ejection of the control produced an overall significant inhibition in its own right ($n = 8$, $P = 0.02$). This was offset by a proportionately greater inhibition in the L-glutamate response caused by calciseptine in comparison to that of the control (Fig. 76 and 77).

Effects of VDCC blockers on the response to SSS stimulation

Barrel blocking was a persistent problem, so the effects of ω -conotoxin GVIA could only be studied in 3 cells. An inhibitory effect was observed in those experiments where ω -conotoxin GVIA was successfully ejected. Calciseptine could only be studied in 1 cell. It was able to inhibit the response following electrical stimulation, suggesting that blockade of L-type VDCCs may also modulate trigeminovascular nociceptive neurotransmission (Fig. 79).

Table 35: Inhibition of L-glutamate evoked firing by VDCC blockers

i) Mean firing rates in response to L-glutamate ejection

VDCC blocking agent	Baseline	Control	Drug
Cadmium	38 \pm 3	34 \pm 3	12 \pm 4 *
ω -Agatoxin IVa	33 \pm 6	27 \pm 6	14 \pm 4 *
ω -Agatoxin TK	39 \pm 7	27 \pm 3	15 \pm 3 *
ω -Conotoxin GVIA	25 \pm 3	22 \pm 2	8 \pm 1 *
Calciseptine	34 \pm 4	23 \pm 3 *	10 \pm 3 *

Action potential firing rates (Hz) of second order neurons in response to ejection of five second pulses of L-glutamate. Five successive pulses were collected for each individual cell and the results for all cells during the three test conditions were pooled to calculate the mean response \pm SEM (* P was calculated < 0.05).

ii) Statistical results

VDCC blocking agent	Drug v. Control	Control v. Baseline
Cadmium	$n = 5, F_{1,4} = 19,$ $P = 0.01$	$n = 5, F_{1,4} = 0.9,$ $P = 0.4$
ω -Agatoxin IVa	$n = 10, F_{1,4} = 12,$ $P = 0.007$	$n = 10, F_{1,1.5} = 3,$ $P = 0.1$
ω -Agatoxin TK	$n = 19, F_{1,4} = 80,$ $P < 0.001$	$n = 19, F_{1,1.7} = 3,$ $P = 0.1$
ω -Conotoxin GVIA	$n = 13, F_{1,4} = 65,$ $P < 0.001$	$n = 12, F_{1,4} = 2,$ $P = 0.2$
Calciseptine	$n = 8, F_{1,4} = 52,$ $P < 0.001$	$n = 8, F_{1,4} = 10,$ $P = 0.02$

F and P values calculated for each treatment group comparing the effects of VDCC blocker and control during co-ejection with L-glutamate. The effect of control ejection on the baseline response was also studied.

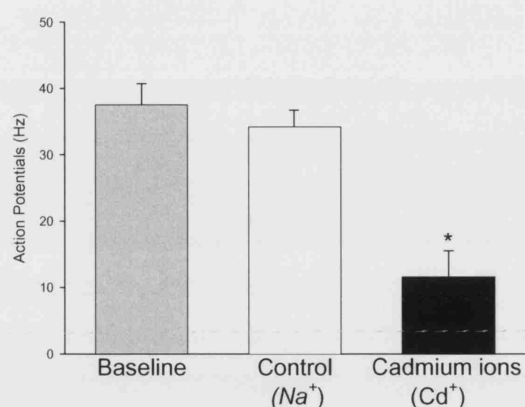


Figure 70: Inhibition of L-glutamate firing by Cd²⁺ ions (pooled data)

Inhibitory action of cadmium ion ejection on the firing rates of trigeminocervical complex neurons following L-glutamate ejection (Pooled data demonstrating the mean \pm SEM values). ($n = 5$, * $P < 0.05$)

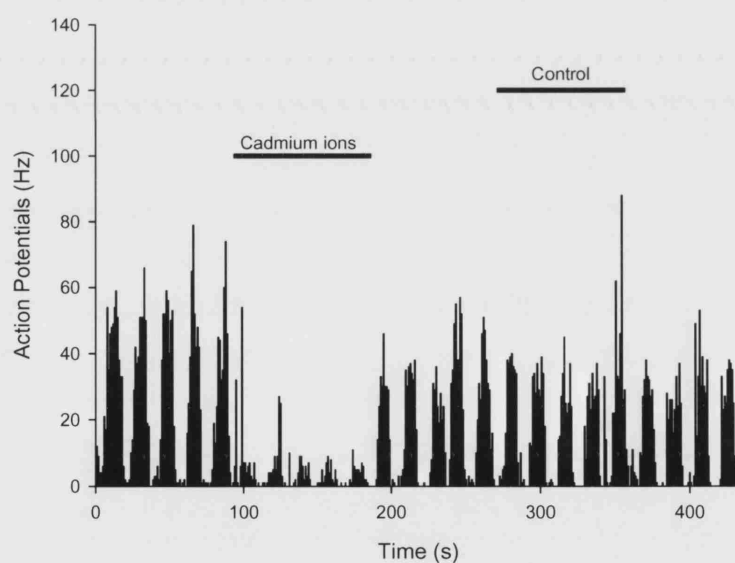


Figure 71: Inhibition of L-glutamate firing by Cd²⁺ ions

In this representative example, cadmium produced a reversible inhibition of the L-glutamate response in excess of any inhibition produced by microiontophoretic ejection of control (sodium ions) at a current of equivalent magnitude, polarity and duration (+20nA, 90s). The inhibition of the L-glutamate response by cadmium ions cannot therefore be attributed to the effect of a current artefact.

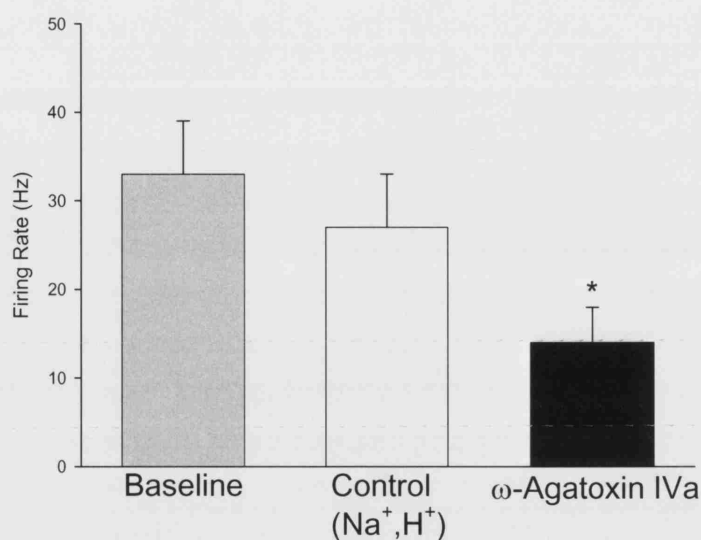


Figure 72: Selective P/Q-type blockade: ω -agatoxin IVa (pooled data)

Ejection of the P/Q-type VDCC blocker ω -agatoxin IVa produced a significant inhibition of L-glutamate evoked firing in comparison to that of control. Mean \pm SEM values are shown. ($n = 10$, * $P < 0.05$)

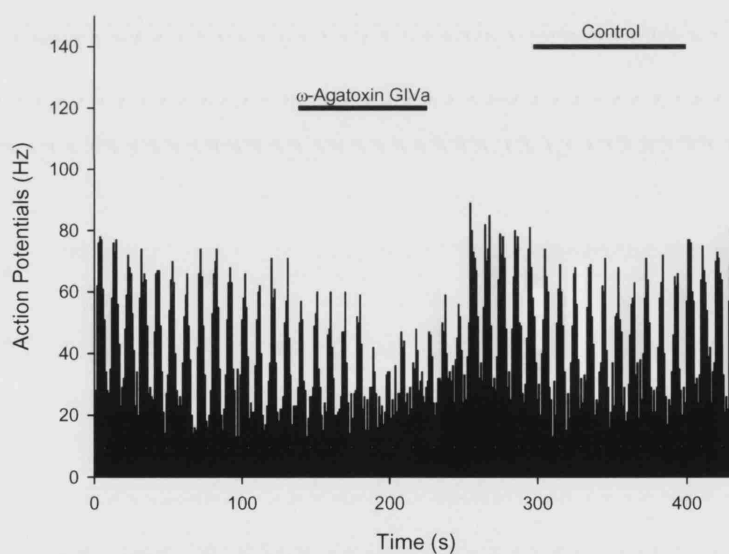


Figure 73: Selective P/Q-type blockade: ω -agatoxin IVa

ω -Agatoxin IVa (P/Q-type) produced a reversible inhibition (see text for discussion) of post-synaptic L-glutamate evoked action potentials. Of note in this example there was a delay in the onset of the inhibition following application of the ejecting current, implying that the response was not solely a result of current ejection. Sodium acetate was the buffer used for adjusting the pH of the agatoxin solution. The control was ejected at an equivalent pH (4) and current (+100nA).

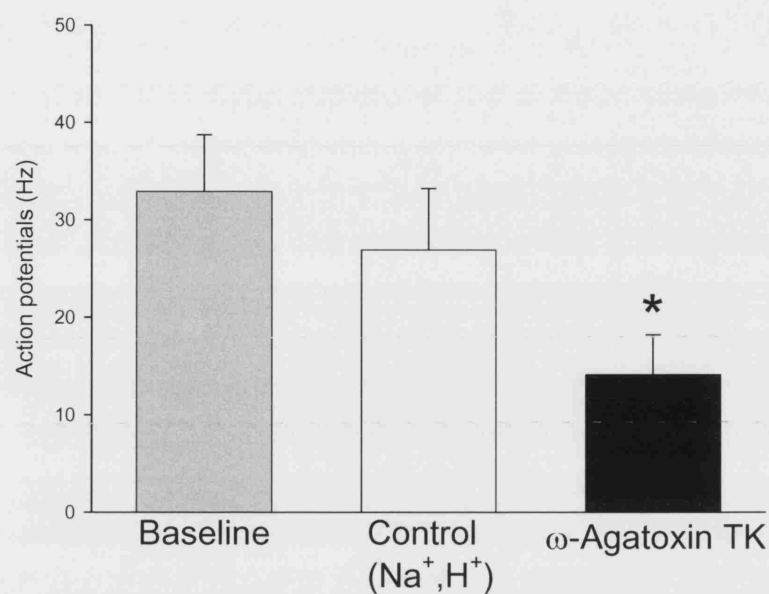


Figure 74: Selective P/Q-type blockade: ω-agatoxin TK (pooled data)

Comparison of the inhibitory effects of ω-agatoxin TK on the response to L-glutamate ejection relative to control (mean ± SEM values are shown). ($n = 19$, $*P < 0.05$)

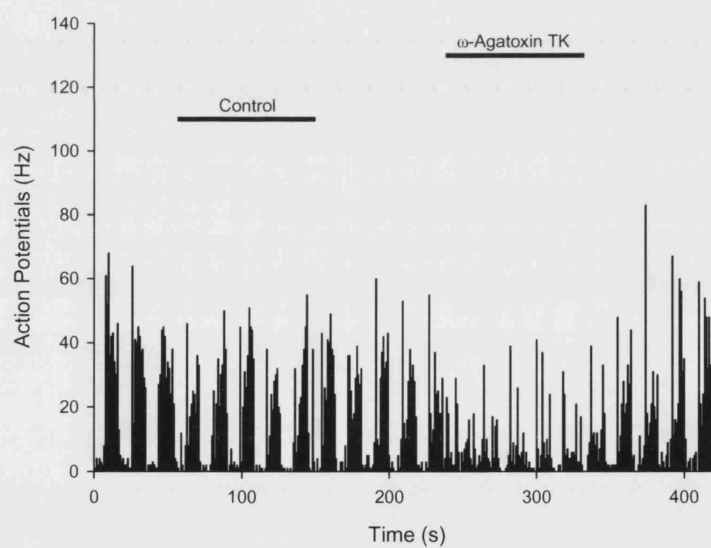


Figure 75: Selective P/Q-type blockade: ω-agatoxin TK

Inhibition of L-glutamate evoked firing by co-ejection of ω-agatoxin TK.

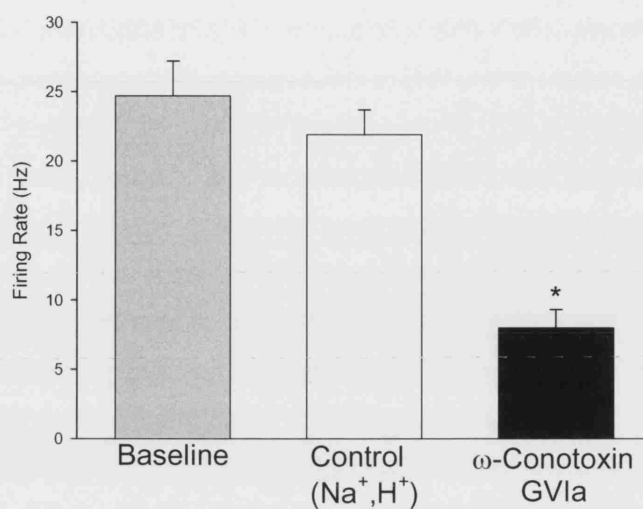


Figure 76: Selective N-type blockade: ω-conotoxin GVla (pooled data)

ω-Conotoxin GVla (N-type VDCC blocker) significantly inhibited the L-glutamate evoked firing rate following microiontophoretic ejection. Mean \pm SEM values are shown. ($n = 13$, * $P < 0.05$)

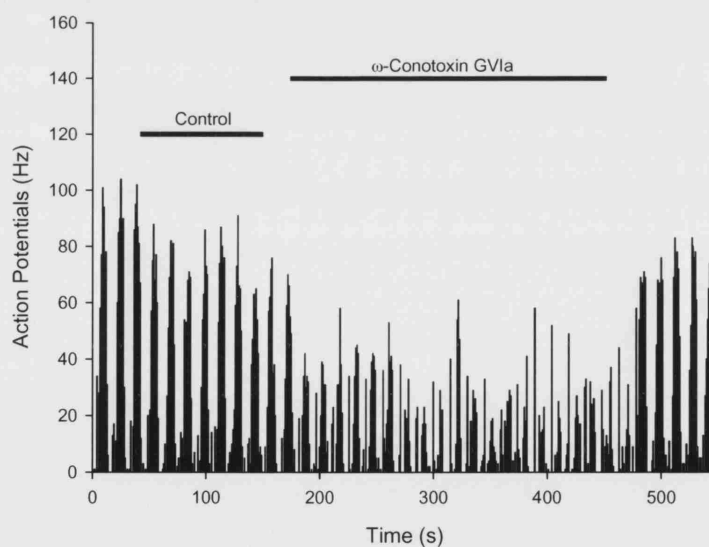


Figure 77: Selective N-type blockade: ω-conotoxin GVla

The N-type VDCC blocker ω-conotoxin GVla produced a reversible inhibition for the duration of its ejection (in this example the two ejection periods were not equal in duration, though for nearly all other experiments they were).

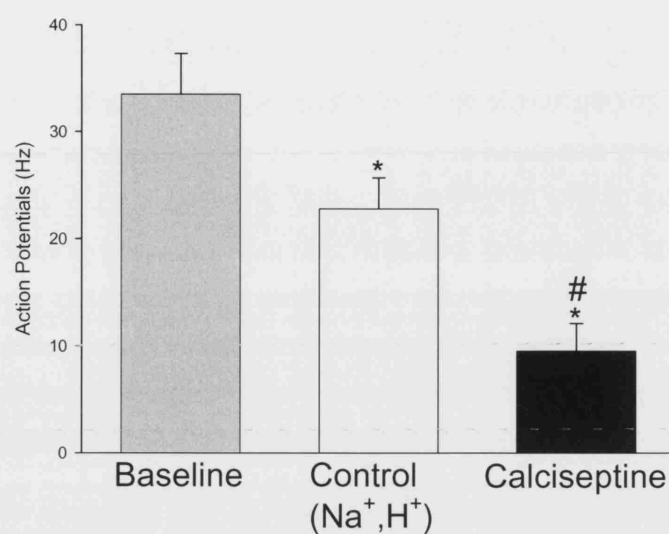


Figure 78: Selective L-type blockade: Calciseptine (pooled data)

The L-type blocker calciseptine also significantly inhibited the neuronal response to L-glutamate above that produced by microiontophoresis of control.

($n = 8$, $^{*}P < 0.05$, * relative to baseline, $^{#}$ relative to control response)

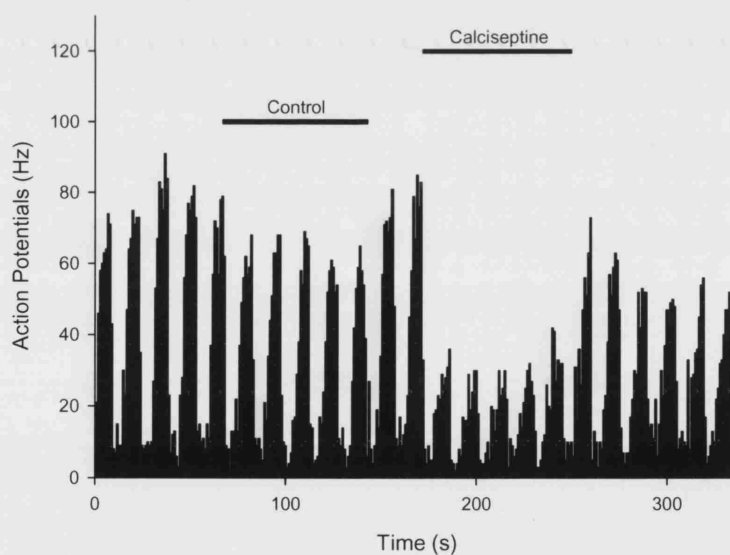


Figure 79: Selective L-type blockade: Calciseptine

Inhibition of L-glutamate evoked firing by ejection of the L-type VDCC blocker calciseptine.

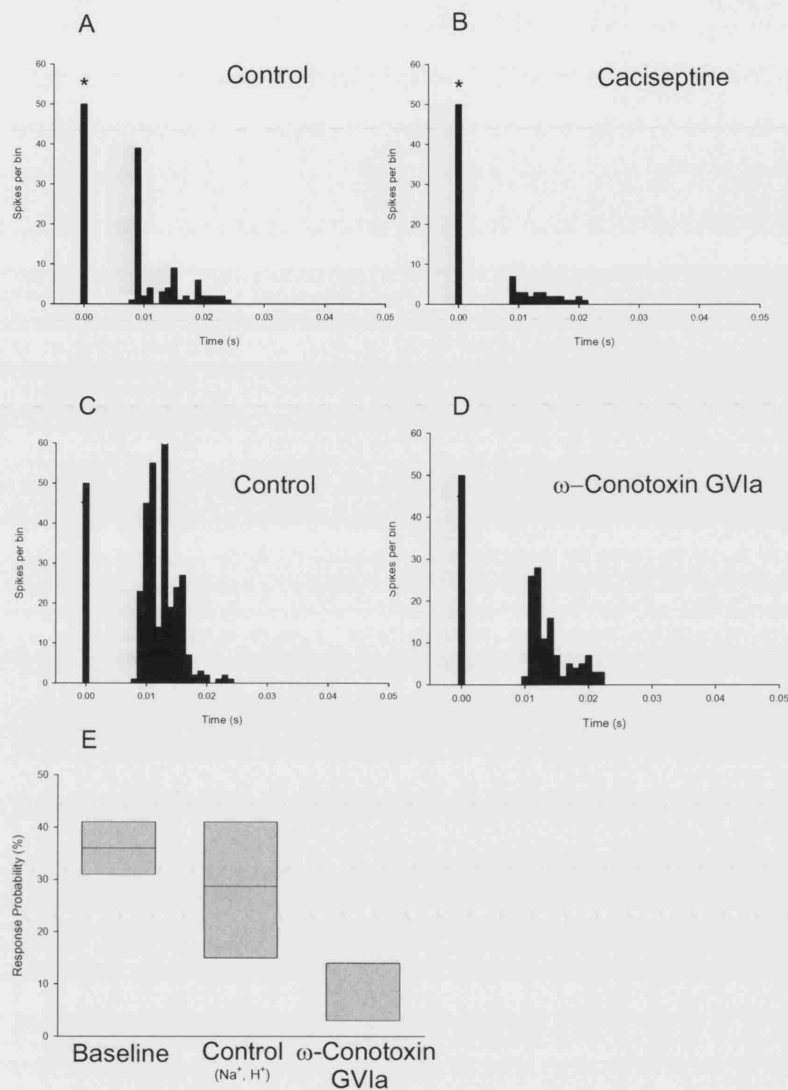


Figure 79: SSS stimulation: effects of VDCC blockers

Post-stimulus histograms demonstrating the inhibitory effect of ω -conotoxin GVIA (C, D and E) and calciseptine (A and B) on the responses of neurons in the TCC following SSS stimulation. This experiment could be performed on only a very few cells (ω -conotoxin GVIA $n = 3$, calciseptine $n = 1$) and so the results are not statistically significant. The median response probabilities with quartile ranges under the three test conditions of the cells treated with ω -conotoxin GVIA are shown in panel E. Barrel blocking however prevented further study. (* stimulus artefact)

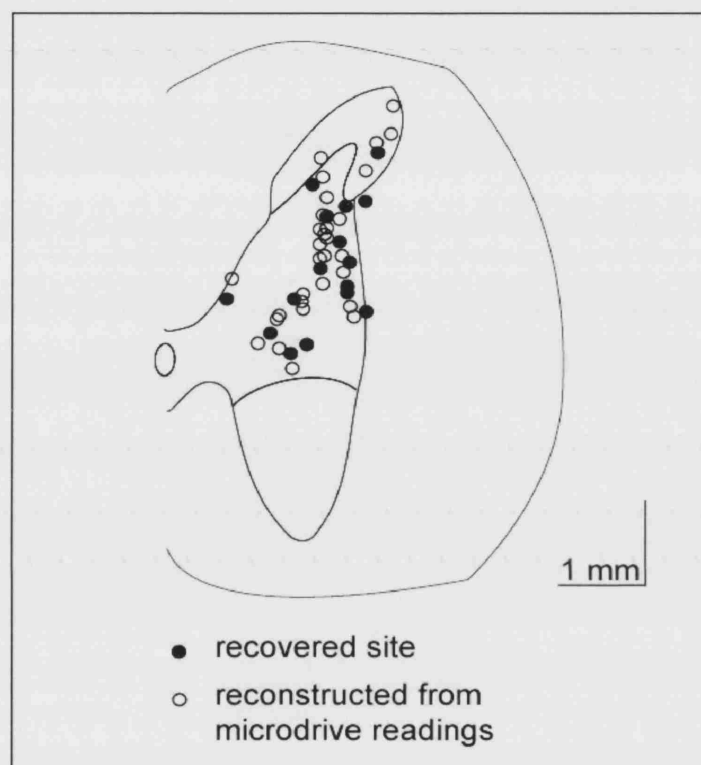


Figure 80: Histological confirmation of recording sites in the TCC

Reconstruction of recording sites using both direct recovery of the marked site, or calculation of the site from marked points (with known depths) along an electrode plunge.

Discussion

This study demonstrates a range of voltage dependent calcium channels (VDCCs) on neurons with input from an intracranial vascular/dural structure located in the trigeminocervical complex of the cat. Most cells were categorised as wide dynamic range on the basis of their receptive field properties; conduction velocities were in the range for A δ fibre neurons. This study demonstrates that L-, N- and P/Q-type channels contribute to neuronal action potential firing in the trigeminocervical complex.

Immunohistochemical studies of the rat dorsal horn demonstrating each of the VDCC subtypes studied here provide supporting evidence for our findings (Chung et al., 2000; Ludwig et al., 1997; Westenbroek et al., 1992; Westenbroek et al., 1998; Westenbroek et al., 1995). There appears to be a differential distribution of VDCCs both regionally and at the neuronal level. The α_{1A} subunit (P/Q type) is found primarily, though not exclusively, on pre-synaptic terminals. The highest density of staining for this subunit is in the deeper laminae (II-VI). N-type α_{1B} subunits are uniformly distributed throughout all laminae of the dorsal horn. Both cell bodies and terminals demonstrate immunoreactivity, although there may be a lower density on the soma. L-type VDCCs appear to be located mainly on cell bodies throughout all levels of the dorsal horn. They also appear to cluster around the bases of major dendrites which is consistent with previous studies in the hippocampus (Westenbroek et al., 1990). Moreover evidence using a model in which the caudal brainstem was bathed locally by ω -agatoxin IVa also supports a role for P/Q channels in the control of dural input to trigeminal neurons (Ebersberger et al., 2004).

As the microiontophoretic transport number (Stone, 1985) for each peptide is not known, and its value can vary in different electrodes, and even different barrels of the same multi-pipette, we cannot quantify our data to assess the relative potency of each compound. It is also not possible to draw definitive conclusions regarding the relative contributions of each of the VDCC channel sub-types to action potential generation under the experimental conditions described. The total flux of compound in a solution produced by a given charge is determined in part by the molecular weight of the

compound and other factors including valency and transport number (Stone, 1985). Smaller quantities of the ω -agatoxin peptides (M.W. approx. 5200) may be ejected by a given charge in comparison to the lighter ω -conotoxin GVIA (M.W. 3037). Thus the importance of a specific VDCC may be underestimated if the appropriate blocking agent is not as amenable to microiontophoretic ejection. Indeed we found that ω -agatoxin IVa was technically more difficult to eject. This disadvantage of iontophoresis is balanced by the precise anatomical localisation that the method affords. As with most methods we view the data in concert with complementary experiments. Studying the trigeminovascular effector junction in the dura mater using intravital microscopy (where the dosing of the test substances can be controlled) has demonstrated a role for each of the L-, N- and P/Q-type VDCCs in neurogenic dural vasodilation (Akerman et al., 2003). These data support a role for each of these VDCCs in the trigeminovascular system.

While the inhibition caused by ejection of cadmium ions was expected to be temporary, we were initially surprised by the finding that inhibition produced by the VDCC peptide blockers was also reversible, and had a short time course. These peptides have a reputation for being irreversible blockers of VDCCs on the basis of *in vitro* experiments. Subsequent studies have demonstrated however that blockade with both ω -agatoxin IVa and ω -conotoxin GVIA can be reversed (Albillos et al., 1994; Ellinor et al., 1994; Regan et al., 1991; Rusin and Moises, 1995). Rapid reversal of blockade by both peptides appears to be strongly voltage dependent *in vitro* (Mintz et al., 1992; Rusin and Moises, 1995; Stocker et al., 1997). Strong depolarisation caused the unbinding of ω -agatoxin IVa to be accelerated by a factor of approximately 5000 (Mintz et al., 1992). This effect was observed in a variety of cells including spinal interneurons. A series of *in vivo* experiments have also demonstrated that the effects of these peptides are reversible, although with a time course of between 10 to 20 minutes (Bowersox et al., 1996; Malmberg and Yaksh, 1995; Nebe et al., 1997; Neugebauer et al., 1996; Sluka, 1997). This is still considerably longer than the rate of reversal observed in these experiments. One important difference is that in previous *in vivo* experiments the duration of exposure of the peptide was much longer. Peptides were allowed to equilibrate with neural tissue for up to an hour prior whereas in our experiments peptides were rarely ejected for longer than 5 minutes. It must also be remembered that only extremely small quantities of peptide are ejected during these

experiments. It may be that changes in membrane voltage caused by the cycles of L-glutamate ejection may hasten the reversal of VDCC block. Once the ejection of toxin is stopped, any residual peptide dislodged from the VDCCs may rapidly diffuse away from the cell, or be inactivated by other means such as by peptidases (Vanegas and Schaible, 2000). Taken together with the parallel evidence for VDCCs on the peripheral end of the trigeminal nerve (Akerman et al., 2003) described above, it is likely that the inhibition observed does reflect an action by the peptides on VDCCs.

The selectivity of ω -agatoxin IVa for calcium channels may be concentration dependent with reduced selectivity reported at high concentrations, while data are lacking for other peptides (Rusin and Moises, 1995). As discussed above it is not possible to know the precise concentration of peptide that the individual neuron under study is exposed to. While the barrel concentration of ω -agatoxin IVa used in these experiments is in excess of those concentrations known to selectively block only P/Q-type VDCCs, in practice the quantity of peptide ejected is likely to be in the femtomolar range (Krnjevic and Phillis, 1963). The problem however remains that we cannot be absolutely certain of the selective (P/Q-type) blocking actions of ω -agatoxin IVa, so that in addition to P/Q-type channels an effect on R-type channel action cannot be excluded. This issue will require further study.

VDCC antagonists have been used clinically as prophylactic agents for the treatment of migraine (Andersson and Vinge, 1990; Silberstein and Goadsby, 2002; Toda and Tfelt-Hansen, 2000). While initially used on the assumption that they would act to prevent contraction of vascular smooth muscle, their precise mode of action in migraine remains unknown. The phenylalkylamine L-type channel blocker verapamil has been used as a prophylactic agent, although the evidence for its effectiveness is very weak (Andersson and Vinge, 1990). Gabapentin (1-(aminoethyl) cyclohexane acetic acid) has also been found to be useful in the management of migraine (Di Trapani et al., 2000; Mathew et al., 2001) in addition to its indication for the treatment of neuropathic pain. It and pregabalin are currently the only agents available which specifically target the $\alpha 2\delta$ subunit of VDCCs. These clinical and experimental observations would suggest that high threshold VDCCs may be targets of some preventive agents, and thus have a role in migraine pathophysiology.

Evidence supporting a role for VDCCs in migraine comes from the finding that FHM in about half of affected families (Estevez and Gardner, 2004) is caused by mutations in the α_{1A} subunit of the P/Q-type channel (Ophoff et al., 1998). When studied *in vitro* all mutated channels have an alteration in their activation kinetics, with a lower threshold of activation a consistent finding (Kraus et al., 2000; Kullmann, 2002; Melliti et al., 2003; Tottene et al., 2002). In comparison with the wild type, FHM P/Q-type channels open more readily at lower membrane potentials offering a gain-of-function effect. Mutation of the CACNA1A gene *in vivo* significantly enhances the release of neurotransmitter at the neuromuscular junction and alters the threshold and velocity of cortical spreading depression (van den Maagdenberg et al., 2004). Whether CACNA1A mutations are more responsible for the aura or the pain symptoms remains however to be determined. Calcium channels may in addition play a role in the brainstem centres responsible for descending modulation of nociception. Functional imaging studies have demonstrated activation of the rostral brainstem, which may include the midbrain periaqueductal grey, in acute episodic migraine without aura (Weiller et al., 1995). Inhibition of P/Q-type channels in the PAG appears to facilitate trigeminal nociception (Knight et al., 2002). Taken together these results support a role for P/Q-, L- and N-type high threshold VDCCs in trigeminovascular nociceptive transmission within the trigeminocervical complex. VDCCs therefore need to be considered in both pathophysiological and potential therapeutic discussions of primary headache disorders.

How then might VDCC be implicated in the pathogenesis of migraine? Calcium conductance is very important in the regulation of neuronal excitability. VDCCs, notably P/Q-type are important presynaptically for the release of neurotransmitters. In the case of the FHM1 R192Q mutation, glutamate release was enhanced at the neuromuscular junction (van den Maagdenberg et al., 2004). They also have important post-synaptic functions. While action potentials are generally initiated in the region of the axonal hillock (Stuart and Sakmann, 1994), the cell membranes of the dendritic tree and soma are not simply passive conducting structures (Hausser et al., 2000; Reyes, 2001; Takagi, 2000). Action potentials are the net result of various ligand- and voltage-gated ionic conductances. Both low- and high-threshold calcium channel mediated currents have been studied in the dorsal horn of the rat (Huang,

1989; Ryu and Randic, 1990; Voisin and Nagy, 2001). As is the case in other neuronal systems, such as cortical and hippocampal pyramidal cells, high threshold calcium currents can generate both regenerative and plateau potentials. Plateau potentials sustained by calcium currents may contribute to the non-linear response properties observed in dorsal horn neurons, permitting resting membrane potential shift towards threshold (Morisset and Nagy, 1998). The firing properties of deep dorsal horn neurons are not therefore rigidly defined. The response of a dorsal horn neuron following nociceptive stimulation is dramatically enhanced following activation of calcium-mediated plateau potentials, probably due to L-type currents (Morisset and Nagy, 1999). Calcium channel conductance is also subject to the modulatory effects of various neurotransmitters and peptides (Murase et al., 1986), which can further affect a neuron's response characteristics at any given voltage. Might episodic fluctuations in calcium conductance lead to abnormal sensory transmission within the trigeminovascular nociceptive system? Under such conditions would otherwise innocuous stimuli be perceived as being painful, as may be the case in the throbbing pain of migraine?

Voltage dependant calcium channels (VDCCs) have provided new insights into the pathogenesis of migraine. The study has demonstrated their role in action potential generation in trigeminovascular nociceptive neurons of the trigeminocervical complex *in vivo*. Voltage-gated channels are an attractive target for elucidating the pathophysiology of migraine. Microiontophoresis provides clear evidence of localisation of these channels within the trigeminocervical complex, and should stimulate further studies of the role of VDCCs in the trigeminovascular system specifically, and in migraine more generally, perhaps indicating a direction for the development of new preventive agents. At present however the systemic side effects of such drugs would probably be intolerable. VDCC blockers such as ziconitide and verapamil have rather unpleasant side effect profiles. Verapamil for example can cause potentially fatal myocardial conduction block. It would therefore be highly desirable if any future VDCC blockers were not to have these systemic effects.

General Discussion

“Life is short, science is long; opportunity is elusive, experiment is dangerous, judgement is difficult. It is not enough for the physician to do what is necessary, but the patient and the attendants must do their part as well, and circumstances must be favourable”.

Hippocrates (460-375 BC)

The present studies have demonstrated that trigeminovascular nociception may be modulated *in vivo* at both second and third order neurons. Action potential firing, driven by electrical stimulation of the superior sagittal sinus (SSS) and local ejection of L-glutamate, could be modulated by microiontophoretic delivery of GABA_{mimetic} drugs, serotonin 5-HT₁ receptor agonists, β -adrenoceptor blockers and blockers of high-threshold voltage dependent calcium ion channels (VDCCs). The fact that acute and preventive migraine therapies modulate thalamic trigeminovascular nociception raises the intriguing possibility that both categories of drugs may exert therapeutic effects at this site.

Superior sagittal sinus stimulation as a model for examining mechanisms of drug action

Conceptualising migraine as a disorder of sensory processing may help to explain many of the symptoms of this complex disorder, including pain, photophobia and phonophobia. While this may be a useful construct for understanding the syndrome, it may paradoxically emphasise the limitations of this model. Nociception is currently the only sensory modality that can be modelled *in vivo*. Activation, or the perceived activation, of trigeminovascular afferents probably accounts for the pain of migraine. These experiments can only examine the modulation of action potential firing by neurons responsive to trigeminovascular nociceptive stimulation. It would be incorrect to claim that this is a model for migraine; however it is probably a reasonable surrogate for the pain component of migraine. This has been validated in studies of trigeminovascular responsive neurons in the trigeminocervical complex (TCC). In this case, modulation of nociceptive neurotransmission is closely correlated with clinical efficacy of acute migraine therapies (Hoskin et al., 1996a; Hoskin et al., 1996b; Storer and Goadsby, 1997). If a dysfunction of sensory processing is capable of generating all of these migrainous symptoms, understanding the pharmacologic modulation of nociceptive neurotransmission, particularly in the thalamus, may offer new insights into the pathogenesis of migraine.

Electrophysiological and functional imaging studies confirm that neurons in the VPM nucleus are activated by intracranial nociceptive stimulation (Davis and Dostrovsky, 1988b; Zagami and Lambert, 1990). All of the neurons examined in these studies received convergent viscerosomatic inputs from cutaneous and intracranial vascular

structures. When VPM neurons are activated by nociceptive somatic stimuli, their response characteristics suggest that they convey sensory discriminative aspects of nociception to the primary somatosensory cortex. It is therefore reasonable to expect that such VPM neurons will be activated during a migraine attack.

Several potential criticisms may be levelled vis-à-vis the nature of the stimulus used to study trigeminovascular neurons and our experimental design. These include:

- a) surgical preparation of the subject may trigger central sensitisation, altering the response properties of TCC and VPM neurons in comparison with intact animals.
- b) electrical stimulation of the SSS is not a physiological stimulus; hence its relevance to the study of headache is questionable.
- c) supramaximal stimulation of the SSS was not used in thalamic studies to activate trigeminovascular afferents - nociceptive afferents may not have been activated at these stimulating voltages, especially as post-stimulus histograms only recorded neuronal responses with latencies of up to 50 milliseconds so that C fibre activity was not specifically examined.
- d) the voltage, but not the stimulating current, was measured during each experiment; this assumed that the resistance of the preparation remained unchanged throughout. If the resistance varied during the course of an individual experiment, the intensity of the nociceptive stimulus would also change.
- e) trigeminal and thalamic neurons rarely responded to nociceptive stimulation of their somatic receptive fields, they are therefore unlikely to be involved in pain transmission.
- f) as more than one cell may have been studied in an individual animal, would the ejection of drugs not affect the behaviour of neurons located subsequently?

Electrical stimulation of the SSS and other dural sites may not be a physiological stimulus, but it remains the only model for which objective, clinical evidence exists confirming its painful nature (Ray and Wolff, 1940). Mechanical stimulation of the dura yields inconsistent results in humans, while application of inflammatory mediators has never been tested.

The fact that second and third order neurons may be activated by electrical stimulation of the SSS and also non-noxious stimulation of their cutaneous receptive fields is largely irrelevant. Convergence of visceral and cutaneous inputs appears to be relatively common at second and third order neurons. There is no evidence to suggest

a link between the nature of the cutaneous receptive field and the visceral input – except that the receptive fields and visceral inputs should be anatomically congruent. It would however have been very interesting to establish whether drug ejection had a differential effect on the vascular visceral versus the convergent cutaneous inputs. If there was a difference it could indicate that different neurotransmitter systems are employed in sensory signalling from the viscera and cutaneous regions.

While it would have been preferable to have measured the current passing through the SSS during each experiment, this was not technically possible. Attempts were made to minimise changes in the electrical resistance of the SSS preparation. In particular the insulating layer of liquid paraffin was regularly replaced and any accumulation of CSF was removed. The order in which the drug and control were ejected was varied, and the post-stimulus histograms were observed to return to baseline values at the end of experiments. It is therefore reasonable to ignore this as a potential confounding variable. Though this is still not ideal, it is no more subject to variations in stimulating intensity than topically applying an “inflammatory soup” to the dura mater (Burstein et al., 1998), a commonly used experimental model.

Supramaximal stimulation was not used in thalamic studies, though it was used in studies on TCC neurons, to reduce the risk of corticothalamic modulation of afferent inputs. Though every effort was taken to minimise current spread from the SSS to the somatosensory cortex (technically it was not possible to elevate the rat SSS on hook electrodes as it was too fragile), it was assumed that some would occur. Corticothalamic neurons modulate thalamocortical activity directly by glutaminergic synaptic neurotransmission, and also indirectly by activation of GABAergic reticular nucleus neurons. A balance had to be achieved between using a stimulating voltage of sufficient magnitude to activate trigeminovascular afferents, while not interfering with thalamic neurotransmission. The stimulating voltage was adjusted to a level just above the threshold value once a suitable unit was identified, though supramaximal stimuli were generally used as a search stimulus. This was felt to be a reasonable compromise as spike morphology and post-stimulus histograms were largely unchanged at this lower voltage.

Central neurons were probably sensitised in the aftermath of surgery and also by electrical stimulation of the SSS. While the sensitising effect of surgery was hopefully minimised by adequate anaesthesia and good surgical technique (though infiltration of local anaesthetic into wound margins could have been performed) prolonged

stimulation of the SSS would undoubtedly have had a sensitising action. This does not invalidate our results, as clinical evidence suggests that sensitisation does appear to occur in a proportion of patients during their migraine headaches (Burstein et al., 2000a). Central sensitisation may be a natural component of a migraine attack and its presence in this model may be desirable.

Even though several cells may have been studied in an individual animal it is unlikely that ejecting a drug into the vicinity of a neuron would have had significant effects on any other cells studied. It is most improbable that the very small quantities of drugs ejected would have been able to diffuse over the distances (which may have been in the order of millimetres in some cases) separating those cells which were studied.

Microiontophoresis as a tool for studying thalamic sensory neurotransmission

Microiontophoresis has been used extensively to study the pharmacological modulation of trigeminovascular neurotransmission in the TCC. There is a strong correlation between an inhibitory action of drugs in this model and clinical efficacy in treating migraine.

Microiontophoresis has not been used previously to study thalamic modulation of trigeminovascular nociceptive neurotransmission, but it is an obvious target for investigation. Despite the limitations associated with this technique, the anatomical precision of drug delivery makes it particularly suitable for studying thalamic sensory pharmacology, especially in comparison with systemic routes of administration. Recording in the thalamus has the additional advantage of suffering from minimal movement artefact, resulting in very stable recording conditions.

Microiontophoretic ejection is suitable for examining the acute effects of drug administration on neuronal activity. This is not a limitation when the drugs, such as naratriptan or even sodium valproate have an immediate, abortive action on migraine headaches. Caution is needed however when interpreting the effects of prophylactic agents. Microiontophoresis cannot mimic the complex changes that undoubtedly occur in receptor density and sensitivity following chronic administration. An entirely different method would be needed to properly evaluate these effects. This

does not invalidate the results of these studies, but it does high-light a limitation of the technique.

Modulation of nociceptive neurotransmission in the thalamus is extremely complex. This is exemplified by the variable results obtained following microiontophoresis of noradrenaline and serotonin in the ventrobasal complex. Both produce facilitatory and inhibitory responses (Andersen and Curtis, 1964; Eaton and Salt, 1989; Phillis and Tebecis, 1967). Noradrenaline however has a purely facilitatory action on afferent excitation on relay neurons of the lateral geniculate nucleus (Rogawski and Aghajanian, 1980), due to activation of α_1 receptors triggering a slow depolarisation. A range of receptors are obviously activated by these neurotransmitters, and they appear to have differing effects depending on the region studied.

These experiments are limited in their scope, concentrating on specific questions of pharmacology relating to drugs with proven therapeutic effects or with a reasonable biological rationale. They were not intended to be an exhaustive examination of monoaminergic modulation of thalamic trigeminovascular sensory processing, as only a limited number of serotonergic and noradrenergic receptors were studied. The lack of intrinsic interneurons in the VPM however allows for a relatively simple interpretation of the results. Ejected drugs can act only at one of two sites: either the pre-synaptic terminals of second order neurons or the cell bodies of third order neurons. When the modulation of L-glutamate driven firing was studied the effect was localised more specifically to the post-synaptic membrane.

The results of the three thalamic studies are summarized in Figure 80. Firing of third order thalamocortical neurons was blocked by agonism of GABA_A, GABA_B and 5-HT_{1A/1B/1D} receptors. Antagonism of β_1 -adrenoceptors had a similar effect, though interestingly isoproterenol did not alter neuronal responses.

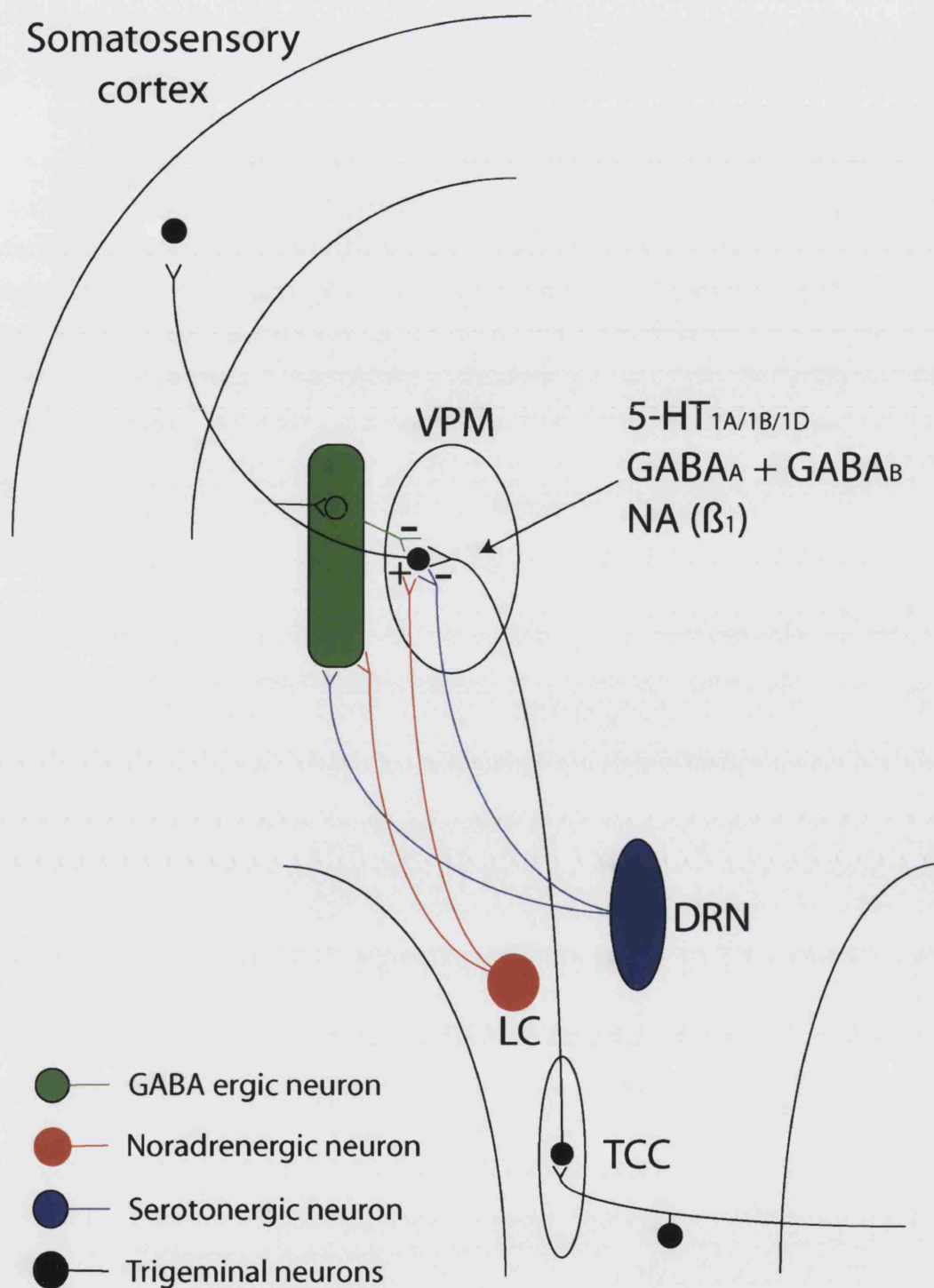


Figure 80: Modulation of trigeminovascular nociception in the VPM: a summary

Agonism of 5-HT_{1A/1B/1D}, GABA_A and GABA_B receptors has a direct inhibitory effect on thalamocortical neuronal firing evoked by electrical stimulation of the SSS and L-glutamate ejection. Antagonism of β₁-adrenoceptors had a similar inhibitory effect. (DRN: dorsal raphe nucleus, LC: locus coeruleus, VPM: ventroposteromedial nucleus of the thalamus, TCC: trigeminocervical complex)

The mechanisms by which GABA_A and GABA_B receptor activation inhibits neuronal firing are well described. Neurons are hyperpolarised by increases in chloride (Cl⁻) and potassium (K⁺) ion conductances, respectively. It was assumed that the inhibition produced by sodium valproate was a result of GABAergic mechanisms; however confirmation would require a clear demonstration that the actions of valproate could be blocked by concurrent ejection of GABA receptor antagonists. Activation of 5-HT₁ receptors also results in membrane hyperpolarisation secondary to an increase in membrane K⁺ conductance. Activation of β -adrenoceptors appears to regulate membrane excitability by more complex means. Agonists of β -adrenoceptors enhance the inward rectifying current I_h (McCormick and Pape, 1990; McCormick et al., 1991). This current has several functions. It is responsible for determining the resting membrane potential of the neuron. It also decreases the response of the thalamocortical neurons to hyperpolarisation, as might occur following activation of GABAergic reticular neurons or interneurons, and is responsible for generating “pacemaker” potentials (Luthi and McCormick, 1998). Antagonism by propranolol of this β adrenergic response could lead to inhibition of thalamocortical neurons. Reduction of I_h may directly inhibit the neuron as blocking it results in hyperpolarisation of the cell’s resting membrane potential. It may also prevent the enhancement of the “anomalous rectification” of I_h that counters the hyperpolarisation of the neuron in response to inhibitory inputs.

Though we were able to demonstrate unequivocally that trigeminovascular nociception could be modulated in the VPM, the question remains whether this would occur at the drug doses used in clinical practice? We cannot unfortunately know the concentration of drug to which each cell was exposed. Though we demonstrated that intravenous naratriptan (5mg/kg) produced a comparable inhibition to microiontophoretically delivered naratriptan, the two results are not equivalent; when given intravenously naratriptan also inhibits trigeminal nociception at first and second order trigeminal neurons. We are faced with the dilemma of being able to deliver a drug with great anatomical precision, but not knowing how much is delivered. This unsatisfactory situation represents the most significant limitation of microiontophoresis.

The results of Study 4 also provide useful insights into the mechanisms of trigeminovascular nociceptive neurotransmission within the TNC. Voltage gated

calcium channels are of interest as VDCC blockers are used clinically as migraine prophylactics and mutations of $\text{Ca}_v2.1$ subunits may be responsible for some cases of FHM. In addition to their central role regulating the exocytotic synaptic release of neurotransmitters, high threshold voltage dependent calcium channels (VDCCs) also play an important function in the control of post-synaptic membrane excitability and integration of synaptic inputs. Unlike previous studies (Ebersberger et al., 2004) which sought to characterise the role of VDCCs in the transmission of sensory information from the dura, microiontophoresis has allowed us to look specifically at the function of high threshold VDCCs on second order trigeminal neurons. Blockade of calcium ion influx in the dendrites and cell body inhibits L-glutamate evoked neuronal depolarisation. More importantly we demonstrated that blockade of VDCCs could inhibit neuronal firing in response to nociceptive stimulation of a dural structure. It is very unfortunate that we could only perform this experiment on a very small number of cells, but it was effective in all those cases where it was successfully carried out. It may therefore be reasonable to suppose that blockers of P/Q-, L- and N-type high threshold VDCCs could be effective anti-migraine agents, providing ofcourse that their side effects were not intolerable.

The thalamus and migraine: synthesis and speculation

We have shown that trigeminovascular nociception may be modulated in the VPM nucleus by pharmacological manipulations of GABA, 5-HT_1 and β_1 -adrenoceptors. Translating these results to a complex biological condition such as migraine is fraught with difficulties. The studies have being confined entirely to the VPM nucleus. As already discussed, many thalamic nuclei are responsible for conveying different components of a painful stimulus. It is not known if modulation of trigeminal nociception in the VPM is the same as in these other nuclei. They also do not address the wider modulatory role of ascending monoaminergic projections on trigeminal sensory processing in the thalamus, such as the functions of other 5-HT or adrenergic receptors particularly 5-HT_2 and α -adrenoceptors, nor the role of monoaminergic inputs to the thalamic reticular nucleus.

Is it possible to draw any inferences on the pathogenesis of migraine from these studies? The most pessimistic response would be that though interesting from a

neuropharmacological perspective, such effects would not be seen at the doses used in clinical practice. It is also pharmacologically unsound to try to study the effects of a preventive agent using this technique.

Though high concentrations were often used in barrels, microiontophoresis ejects only minute quantities of each drug (in the femtomolar range). While we cannot be sure of the concentration of drug to which each cell is exposed, it is unlikely to be so high that our findings would be entirely invalidated. Also while microiontophoresis may not be entirely appropriate for examining the effects of prophylactic agents, we have demonstrated a locus of action for such drugs in the thalamus. At the very least this certainly warrants further study.

If we think of migraine purely in terms of activation of trigeminal afferents and pain, the answer is again probably no. Though the flow of trigeminovascular nociceptive information may be modulated in the thalamus, it does not necessarily provide any information on the mechanisms of trigeminal activation.

Migraine is however not just a disorder of head pain - it may be a disorder of sensory processing, with pain being a major clinical feature. Other symptoms, particularly photophobia and phonophobia, are also essential for the diagnosis of migraine. These and other symptoms may be present between and during migraine attacks. It suggests that migraineurs' perception of their sensory environment is different to that of non-migraineurs, and that it may fluctuate according to the "migrainous state" of the brain. Electrophysiological evidence tends to support this view. It is more likely that any such sensory dysregulation would occur in the thalamus/cortex (in practical terms the thalamus and cortex should be considered as a single functional entity) than at more peripheral sites. The descending modulatory action of brainstem centres has been examined in detail and a potent anti-nociceptive action has been demonstrated, mediated in part by activation of serotonergic receptors. Injection of naratriptan in the ventrolateral periaqueductal grey inhibits second order trigeminal neuronal firing following nociceptive stimulation of the dura mater (Bartsch et al., 2004). This demonstrates that pharmacological manipulations of brainstem structures may have significant effects on nociceptive neurotransmission in distant sites within the CNS.

The studies in this thesis indicate that pharmacologic modulation of thalamocortical neurons may significantly inhibit action potential firing in response to nociceptive stimulation. What if the opposite was also true – that action potential firing could be facilitated by modulatory, particularly monoaminergic, inputs? A growing body of

evidence indicates that the rostral brainstem may be implicated in the pathogenesis of migraine. This region contains the dorsal raphe nucleus and the loci coerulei. These monoaminergic nuclei are critical for arousal and attention. During arousal they alter sensory processing in the thalamus by changing the firing of VPM neurons from burst to tonic mode. They may also have ascending anti-nociceptive functions. Could abnormal activity in these regions possibly facilitate transmission of sensory information in the thalamus? Are the symptoms of migraine a product of a central sensory dysregulation arising from abnormal serotonergic and catecholaminergic neurotransmission? Perhaps triptans and β -blockers exert some of their therapeutic effects by inhibiting this facilitated transfer of sensory information through the thalamus. This may also explain why they do not have general analgesic properties.

Valproate alternatively modulates thalamic sensory neurotransmission by augmenting GABAergic inhibition from the reticular nucleus. GABAergic hyperpolarisation of thalamocortical cells renders them more resistant to depolarising inputs from any source (see Fig. 81)

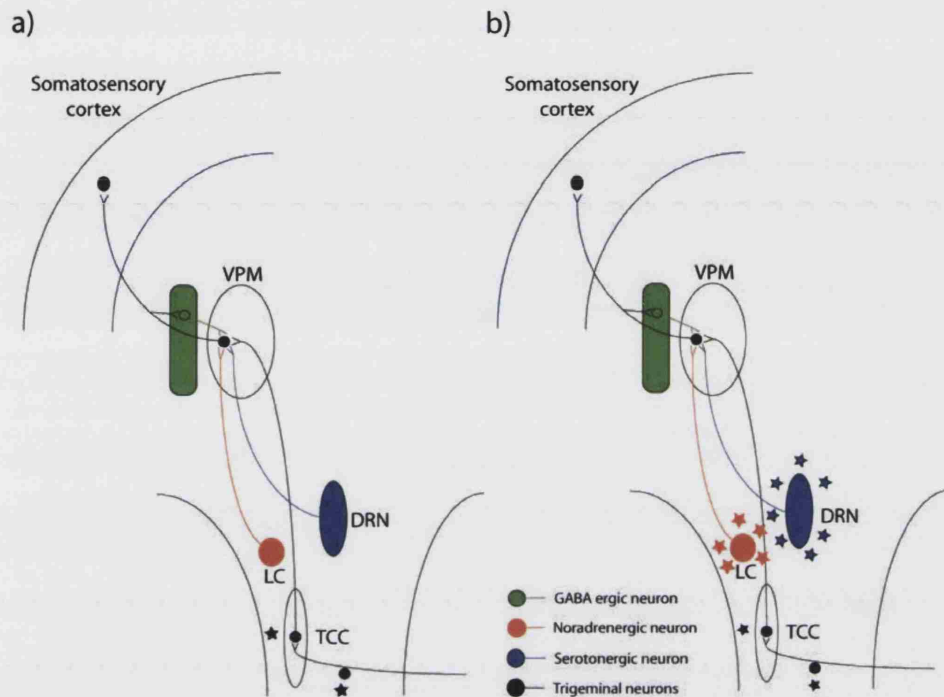


Figure 81: A speculation on the pathogenesis of migraine, and mechanisms of drug action

Proposed model for monoaminergic facilitation of trigeminovascular nociception in the VPM nucleus of the thalamus.

a) trigeminal sensory information is relayed to the primary somatosensory cortex through the trigeminocervical complex (TCC) and the VPM nucleus.

b) the VPM nucleus receives monoaminergic inputs from the locus coeruleus (LC) and the dorsal raphe nucleus (DRN). Abnormal activity in the LC and the DRN may modulate the transmission of trigeminovascular afferent information in the VPM nucleus.

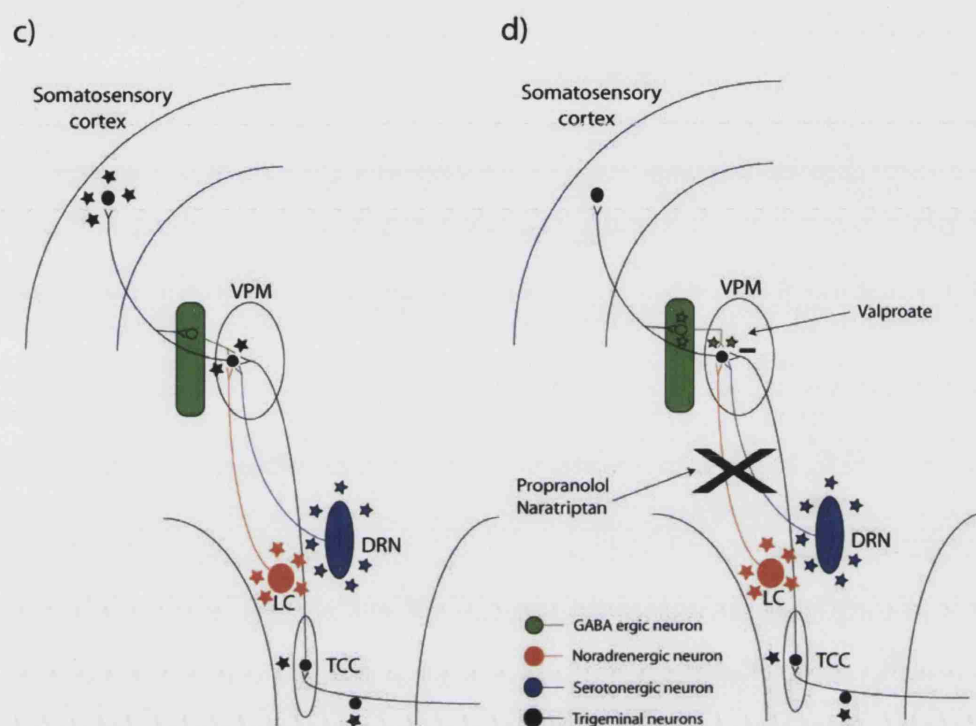


Figure 81 (cont.)

c) abnormal monoaminergic modulation of thalamocortical neurons leads to abnormal action potential firing of cortical neurons. This is perceived as pain and may account for the other sensory symptoms of photo- and phonophobia if extended to other thalamic relay nuclei.

d) sodium valproate enhances GABAergic hyperpolarisation of thalamocortical neurons, inhibiting the abnormal activation of cortical neurons. Naratriptan inhibits the abnormal serotonergic modulation of thalamocortical neurons by activating 5-HT_{1A/1B/1D} receptors on these cells. This results in a K⁺ dependant hyperpolarisation of the cell membrane. Propranolol also causes membrane hyperpolarisation by antagonism of β_1 -adrenoceptors with presumably modulation of I_h. This counters the effects of abnormal modulatory inputs from the locus coeruleus.

Future Research

These studies have provided a tantalising insight into the complex mechanisms of sensory modulation within the thalamus. Many questions however remain to be answered; these may include some of the following:

The rat VPM nucleus does not contain intrinsic interneurons; can these findings be replicated in higher mammals, such as the cat or primates, whose thalami do contain such GABAergic cells?

What is the wider role of the brainstem monoaminergic nuclei in modulating sensory neurotransmission in the thalamus? Serotonin 5-HT₂ receptors are implicated in the triggering of migraine headaches, what role do they and 5-HT₃ receptors have in the facilitation of sensory transmission and specifically of trigeminovascular neurotransmission? It would be interesting to see how activation of the locus coeruleus and dorsal raphe nucleus modulates trigeminovascular nociception, especially given that they also innervate the thalamic reticular nucleus. This could be done either electrically or by local injection of excitatory amino acids into these nuclei.

Can the effects of preventive drugs not be studied more effectively? These studies are not possible using microiontophoresis and would require surgical implantation of chronic recording electrodes, such as microwires. After an adequate recovery period baseline recordings would be made. Neurons would be activated by nociceptive stimulation of the face, or possibly by a dural electrode implanted in the skull. Drugs could then be given systemically (e.g. by intraperitoneal injection), or locally into the thalamus by an indwelling catheter and the responses studied after a period of chronic drug administration. This should preferably be after a period of several months to more accurately reproduce the dynamic changes in receptor function which would probably have occurred.

It is assumed that other triptans will also be able to inhibit trigeminovascular nociception in the thalamus, but is this the case? Studies will have to be performed to verify this.

Central dopaminergic neurotransmission has also been implicated in the pathogenesis of migraine. Does dopamine also modulate trigeminovascular neurotransmission in the thalamus?

Conclusion

Microiontophoresis offers a means of studying the effects of drugs on individual or small numbers of cells *in vivo*. The anatomical precision of delivery therefore makes it ideally suited for studying the pharmacology of trigeminovascular neurotransmission in the trigeminocervical complex (TCC) and the thalamus.

Trigeminovascular nociception may be inhibited in the felid TCC by blockers of P/Q-, L- and N-type high threshold voltage gated calcium channels (VDCCs). VDCCs may therefore be suitable targets for the development of novel anti-migraine drugs.

Sensory information from the cranio-facial region is relayed to the primary somatosensory cortex from the ventroposteromedial nucleus (VPM) of the thalamus. Trigeminovascular nociception may be modulated in the VPM of rats by naratriptan, sodium valproate and propranolol. This suggests that the thalamus may be a target for both preventive and abortive anti-migraine drugs.

Glossary

AC: adenylate cyclase

ACh: acetylcholine

AMPA: α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid

C: cervical vertebra (number) or corresponding segment of the cervical spinal cord

Ca²⁺: calcium ions

CBF: cerebral blood flow

Cd²⁺: cadmium ions

CGRP: calcitonin gene-related peptide

Cl⁻: chloride ions

CM-Pf: central median-parafascicular nucleus of the thalamus

CNS: central nervous system

CNV: contingent negative variation

CSD: cortical spreading depression

DHE: dihydroergotamine

DRN: dorsal raphe nucleus

EEG: electroencephalogram

EPSP: excitatory post-synaptic potential

FHM: familial hemiplegic migraine

fMRI: functional magnetic resonance imaging

GABA: γ -amino-butyric acid

H⁺: hydrogen ions

HCl: hydrochloric acid

HCO₃⁻: bicarbonate ions

HRP: horseradish peroxidase

5-HT: serotonin (5-hydroxytryptamine)

IDAP: intensity dependence of auditory evoked potentials

ISA: intrinsic sympathetic activity

i.v.: intravenous

K^+ : potassium ions

LC: locus coeruleus

LT: light touch

MA: migraine with aura

MEG: magneto-encephalography

MDH: medullary dorsal horn

MMA: middle meningeal artery

MO: migraine without aura

mRNA: messenger ribonucleic acid

NA: noradrenaline

Na^+ : sodium ions

NH^2 : amine

NH_3^+ : amide ions

NKA: neurokinin A

NMDA: *N*-methyl-*D*-aspartic acid

NO: nitric oxide

NRM: nucleus raphe magnus

NS: nociceptive specific

OH^- : hydroxide ions

PAG: periaqueductal grey

PET: positron emission tomography

PPE: plasma protein extravasation

PO: by mouth

Po: posterior nucleus of the thalamus

PSH: post-stimulus histograms

REM: rapid eye movement

RF: receptive field

RT: thalamic reticular nucleus

SI: primary somatosensory cortex

SII: secondary somatosensory cortex

Sm: nucleus submedius of the thalamus

SP: substance P

SRT: spinoreticular tract

SSN: superior salivatory nucleus

SSRI: selective serotonin reuptake inhibitor

SSS: superior sagittal sinus

STT: spinothalamic tract

$t_{1/2}$: plasma concentration half-life

TCC: trigeminocervical complex

TMS: transcranial magnetic stimulation

TNC: trigeminal nucleus caudalis

V_{I/II/III}: trigeminal nerve: ophthalmic, maxillary, mandibular divisions

VB: ventrobasal complex of the sensory thalamus

Vc: caudalis division of the spinal trigeminal nucleus

Vi: interpolaris division of the spinal trigeminal nucleus

Vo: oralis division of the spinal trigeminal nucleus

Vp: principal trigeminal nucleus

Vsp: spinal nucleus of the trigeminal nerve

VDCC: voltage dependent calcium channel

VL: ventrolateral nucleus of the thalamus

VMpo: ventral medial (posterior) nucleus of the thalamus

VPI: ventroposterior inferior nucleus of the thalamus

VPL: ventroposterolateral nucleus of the thalamus

VPM: ventroposteromedial nucleus of the thalamus

VPMpc: parvicellular portion of the ventroposteromedial nucleus

WDR: wide dynamic range

IX: glossopharyngeal nerve

X: vagus nerve

ZI: zona incerta

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